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The Fate of an Intravenously Injected Dye (Brilliant Vital Red) with Special Reference to its Use in Blood Volume Determination[1]

By H. P. Smith

From the Department of Pathology and Bacteriology, Johns Hopkins University

THE experimental introduction of dyes into the blood stream is an old procedure and many of them have been tested in this manner. In most instances the object has been to stain vitally certain of the body tissues as an aid to histological study, and only rarely were any observations recorded concerning the amount of dye in the blood stream. The latter phase has received attention only from those interested in the determination of blood volume. This application was first made by Keith, Rowntree and Geraghty (1) who injected a colloidal dye—vital red—the amount of plasma in the body being estimated by noting the extent to which the injected dye was diluted by the circulating plasma. This dye and others having similar properties have been used by many investigators since that time, but the work of Dawson, Evans and Whipple (2) is the first in which a large series of dyes were compared under uniform conditions. These authors presented in some detail curves of dye elimination from the blood stream. Many dyes were found to disappear from the blood stream quite rapidly; others, like brilliant vital red, much more slowly. All who have used vital red have observed that the dye is lost from circulation partly within the first few hours and more or less completely after the lapse of several days. However, samples of plasma taken 4 or 5 minutes after injection of the dye contain very little more dye than others taken 5 or 10 minutes later. Very few efforts have been made to study in detail and over long periods of time the rate and mode of elimination of the dye from the circulation and almost no attempts have been made to determine its subsequent fate. The main purpose of the following experiments was to study the fate of the dye from the time it was injected into the blood stream until it was finally eliminated from the body. Such studies are not only essential for the proper interpretation of blood volume figures, but also shed some light upon the fundamental processes of lymph formation and of phagocytosis. No doubt many substances occurring normally in the body are handled by it in a somewhat similar manner. Although there has been

[1] Submitted for publication June, 1924.

much speculation about the phagocytic activity of the "reticulo-endothelial system" of Aschoff, still most of the evidence on this subject is based purely on morphological findings and hence is qualitative in nature. The method outlined below permits the quantitative study of these problems of phagocytosis from the physiological point of view.

Elimination of brilliant vital red from the body. After intravenous injection of large amounts of the dye the plasma remains colored for several days, though ultimately it again becomes dye-free. A number of experiments were performed to determine its mode of exit from the body, and after establishing the mode of excretion, we shall return to consider its behavior in the body prior to such elimination.

Adult dogs were used in all experiments. In certain of the cases the amount of dye injected was the same as that used for the ordinary blood volume determinations (3 mgm. per kilo). In other cases as much as 180 mgm. per kilo were injected.[2] In making up the dye solution for injection two grams were added to 100 cc. of water. Since the dye specimen contained a small amount of an insoluble red pigment, in the final filtered solution there remained only 1.5 grams of the soluble brilliant vital red, but this soluble portion of the dye was found to be quite satisfactory for the blood volume work. It leaves the circulation rather more slowly than most of the samples which have been used previously.

In no case was any of the dye found in the urine, and although the urine did in some cases become more yellow than normal, still this yellow color in no way suggested the red tinge which would have been imparted by even a very minute amount of the dye. It is unlikely that it represents a decomposition product of the dye, for the latter is very stable chemically, and is unaffected by acids and alkalies except in relatively high concentration. It seems most likely that the yellow color is due merely to an excess of the yellow pigments normally found in the urine, although specific tests have not been employed. Nevertheless, it is worthy of note that the excess of yellow color was seen only in prolonged experiments in which large amounts of dye had been injected on successive days. In these experiments the plasma also was slightly more yellow than normal. This could be observed in making colorimetric estimations of the amount of dye in the plasma. Nevertheless, this yellow color in the plasma was never so great as to interfere seriously with the dye readings.

Since the dye is not eliminated in the urine, attention was next directed to the possibility of elimination by way of the liver. Relatively few experiments were made upon this phase of the subject, although in several cases the common bile-duct was cannulated and dye was then injected into the blood stream. In such experiments the dye appeared in the bile within the course of a few minutes and in a relatively concentrated condition. Quantitative determination of the dye in the bile is difficult unless it is present in such large amounts that it can be compared with the standard

[2] The dye used in these experiments was obtained from the National Aniline and Chemical Company.

after the addition of large amounts of water to dilute the bile pigments. It is also necessary to add small amounts of normal dye-free bile to the standard in order that the colors may be more alike qualitatively. In one experiment the bile was estimated to contain fifteen times as much dye as an equal volume of plasma taken at the same time. In this case both the bile and plasma samples were taken about an hour after the injection of the dye. In another experiment a permanent bile fistula was made by the method recently outlined by Rous and McMaster (3). Twenty cubic centimeters of dye were injected intravenously and the bile was collected over a period of about 36 hours. Within an hour the dye was present in the bile in concentration several times as great as in the plasma. At the end of 24 hours the dye in the plasma had decreased to about half of its original concentration and the bile still contained definite amounts of it. A few hours later the bile contained such small quantities of dye that it was impossible to make any quantitative estimation on account of the interference from the bile pigments. Within 36 hours the quantity of dye in the bile was so small that it was not clearly recognizable in the presence of these pigments, although undoubtedly still occurring in small amounts. It was estimated that in the whole 36 hours not more than one-fourth of the dye injected had been eliminated in the bile. Nevertheless, the plasma now contained only about 10 per cent of its original content of dye. In an attempt to avoid the necessity of making a biliary fistula for the study of its elimination by the liver it was thought that the dye might be recovered from the feces. But when the feces were examined, no dye whatever could be found regardless of the amount injected into the blood stream. It seemed that the dye was somehow destroyed in the intestinal tract or possibly absorbed from it. In a series of dogs enormous quantities of dye were given by stomach tube and in these cases the feces never showed the faintest trace of dye either on direct inspection or in aqueous extracts. Other experiments were performed in which the dye was injected by means of a small needle and syringe into a loop of upper jejunum exposed by laparotomy. Again, the feces showed no dye. In one experiment the dye was injected into the transverse colon. A bowel movement on the second day showed a small amount of dye, not over 4 or 5 cc. although 20 cc. had been injected. In all of these experiments samples of blood were taken and centrifugalized, but no dye was found in the plasma and the tissues never showed the faintest trace. It would seem, then, that dye absorption by the intestine could be ruled out. It was not ascertained whether the dye is actually destroyed in the intestinal canal or whether it is "adsorbed" or otherwise taken up by bacteria or other particles in the feces. If so, it is so firmly held that aqueous extracts of the feces contain no demonstrable dye. Bearing on this point are several experiments performed by Dr. E. L. Burky of this laboratory. Intestinal organisms were transplanted into sterile bouillon containing brilliant vital red in solution. It was readily seen

that much of the dye was removed from solution; it appeared in the bacterial sediment which settled to the bottom of the flask in the course of several days. It may be that similar binding of the dye occurs in the intestinal tract and that in the presence of the large amount of solid material the dye gives no perceptible coloration.

Thus it would seem that none of the dye can be detected in the urine and within the course of the first day not over 25 per cent of the total amount appears in the bile. Nevertheless, the concentration of dye in the blood stream is then only a small fraction of its original value. Careful search failed to reveal any other important method of elimination from the body. There is no evidence that any is excreted into the intestinal tract. Nowhere is the mucosa of the alimentary tract stained by the dye even when large doses of the dye are given. Nor does it appear to be excreted along with the cutaneous excretions, for repeatedly attempts have been made to recover it by washing the skin with water, alcohol and ether, but the washings have never contained any demonstrable dye.

The staining of the tissues. The loss of the dye from the circulation is evidently largely due to its being taken up and stored by certain tissues. After repeated injections or after single large injections the tissues gradually become quite red in color. There seems to be no *a priori* reason why the distribution of brilliant vital red may not be similar to that of many other vital dyes which have been studied by numerous investigators. A few experiments confirmed the supposition. In these relatively large doses were injected intravenously so that the tissues might be deeply stained and the microscopic recognition of the dye would be easy. In some of the experiments a single injection of about 50 mgm. per kilo was made but most of the animals were injected on successive days until amounts varying from 50 to 200 mgm. per kilo had been given. One or more days after the last injection the animals were killed. All showed a similar distribution of the dye. The skin and connective tissues in general were quite red. The lymph nodes were of the normal size but on section the central two-thirds of each node was of a bright red color. The peripheral part was slightly pink also, but not so deeply stained as the central portion. The spleen and liver were rather more of a purple color than usual. Microscopic study of the tissues is accompanied by considerable difficulty on account of the solubility of the dye in water and other solvents. But if frozen sections are made without preliminary fixation and examined at once, the distribution of the dye can be followed quite easily. The dye is taken up in great quantities by the Kupffer cells of the liver but none whatever can be seen in the liver cells themselves. This is remarkable in view of the fact that the dye passes into the bile. Large amounts of it were also seen in the reticulum cells of the spleen and lymph nodes and in the macrophages of the body tissues generally. None of the dye was seen anywhere in the mucosa of the gastrointestinal tract. Many of the convoluted tubules of the kidney contained great quantities of the dye

stored in the form of large intracellular granules, the appearance resembling that seen after the intravenous injection of carmine. Marshall and Vickers (4) have recently described similar accumulations of dye in the renal tubules after intravenous injections of phenolphthalein. Their work goes to show that this dye is passed on into the lumina of the tubules by these cells. Aschoff (5), however, maintains that the dye found in the renal cells has been absorbed by them from the lumina of the tubules where it arrived by way of the glomeruli. If the latter theory obtains in the case of brilliant vital red, it is evident that the absorption is quite complete, for none appears in the urine as in the case of phenolphthalein or carmine.

It seems that the dye is not taken up by the leukocytes of the blood stream. Its absence is shown in smears taken by puncture and from cells taken from centrifugalized samples of blood and examined both in smears and in thin moist coverslip preparations. There is no evidence that any of the tissues, with the possible exception of elastic tissue, take up the dye diffusely. In dogs stained with enormous quantities of the dye it is sometimes seen that the elastic tissue, particularly of the aorta, is pink. This intra-vitam staining of elastic tissue has also been noted by Petroff (6).

To summarize: brilliant vital red is eliminated from the body quite slowly and the elimination is brought about largely by the liver. Prior to elimination the dye is removed in part from the blood stream and stored by the tissues, chiefly in the body phagocytes and in the epithelial cells of the kidney.

Expt. 69. Elimination of brilliant vital red from the blood stream following injection of a small amount of the dye (see Charts 1 and 2).

Collie-airedale mongrel, male. Weight 14 kgm.

July 30. Intravenous injection of 5 cc. of 1.5 per cent brilliant vital red. Samples of blood were taken at frequent intervals thereafter and from each tube of blood a small amount of plasma was taken and diluted with two parts of saline. This unknown was then compared in a colorimeter with a standard dye solution prepared by diluting 5 cc. of normal dye-free plasma with 5 cc. of saline, and 5 cc. of a dye solution prepared by diluting 0.75 cc. of the 1.5 per cent dye solution to 200 cc. with water.

The dye readings recorded below and in Charts 1 and 2 are corrected for the oxalate solution added to prevent coagulation of the blood.

Four minutes after the injection the dye reading of the plasma was 177 per cent of the standard; after 1 hour 162 per cent; after 3 hours 139 per cent; after 6 hours 115 per cent; after 9 hours 100 per cent; after 12 hours 84 per cent; after 15 hours 73 per cent; after 18 hours 63 per cent; after 21 hours 57 per cent; after 24 hours 49 per cent; after 27 hours 41 per cent; after 30 hours 35 per cent; after 48 hours 24 per cent; after 72 hours 18 per cent.

Expt. 70. Elimination of brilliant vital red from the blood stream following injection of a small amount of the dye (see Charts 1 and 2).

Male shepherd. Weight 16 kgm. Technique similar to that in Exp. 69.

July 30. Intravenous injection of 5 cc. of 1.5 per cent solution of brilliant vital red.

Four minutes after the dye injection the dye reading of the plasma was 175 per cent of the standard; after 1 hour 150 per cent; after 3 hours 120 per cent; after 6 hours 97 per cent; after 9 hours 80 per cent; after 12 hours 62 per cent; after 15 hours 51 per cent; after 18 hours 43 per cent; after 21 hours 38 per cent; after 24 hours 34 per cent; after 27 hours 33 per cent; after 30

hours 31 per cent; after 48 hours 17 per cent; after 72 hours 11 per cent.

Experiments 69 and 70 (see also Charts 1 and 2) show that at first the dye is eliminated from the blood stream much more rapidly than later on. In fact, after the lapse of 24 or 48 hours the concentration of dye in the blood stream has become almost constant, although there is still a considerable amount of it in circulation. There is every reason to believe that the phagocytes have reached an equilibrium with the dye-tinged plasma. That the phagocytic reaction is reversible is indicated by the fact discussed below that eventually all of the dye is eliminated from the body, and the tissues once more become pale. At no time before completion of this elimination process does the plasma become entirely free from dye. In fact the plasma and the phagocyte seem to lose their dye simultaneously and neither becomes entirely dye-free until the last of the dye has been eliminated from the body. The state of equilibrium between dye-laden phagocytes and dye-colored plasma is shown in a number of experiments described in greater detail below. In these it was proved that the dye mixes to some extent with the lymph but that lymph from certain of the larger lymphatics contains almost no dye for many hours, a result of the fact that the phagocytes in these tissues remove almost all of the dye from the lymph before it reaches the larger lymph vessels where it is collected for examination. If, however, one waits several days after injection of the dye, it will then be found that such lymph contains considerable quantities of dye just as does the plasma. The presence of the dye now is due to the fact that the phagocytes of these tissues have reached an equilibrium with the dye-tinged fluids which surround them, so that lymph passes by them without losing its content of dye.

Expt. 53. Elimination of dye from blood stream of a dog which had received previous injections of dye (see Chart 1).

Male bull dog. Weight 17 kgm.

July 17 to 22 inclusive: Injection daily of 20 cc. of 1.5 per cent solution of brilliant vital red. August 8, injection of 7.5 cc. more of the dye solution. As indicated below and in the chart, samples taken 3 and 4 days later show at that time a very slow rate of elimination of dye from the blood stream.

August 12. Five cubic centimeters of dye were injected. Samples of blood were then taken at frequent intervals. One part of plasma from each tube was diluted with two parts of saline and compared colorimetrically with a standard made by diluting 5 cc. of normal dye-free plasma with 5 cc. of saline and 5 cc. of a dye solution prepared by diluting 0.75 cc. of the 1.5 per cent brilliant vital red with water to 200 cc. The readings were corrected for the oxalate solution added to prevent coagulation.

The following results are given graphically in Chart 1. On August 11, the plasma contained 75 per cent dye; on August 12, just before the injection of more dye the reading was 65 per cent; 4 minutes after injection, 254 per cent; 1 hour, 211; 3 hours, 194; 6 hours, 169; 9 hours, 136; 12 hours, 128; 15 hours, 118; 18 hours, 105, 21 hours, 98; 24 hours, 87; 27 hours, 84; 30 hours, 80; 52 hours, 75; 76 hours, 65; 100 hours, 61.

Expt. 63. Elimination of dye from blood stream of dog which had had previous injections of dye (see Chart 1).

Male mongrel terrier. Weight 11 kgm. Technique similar to that in Exp. 53.

The following results are given graphically in Chart 1. On August 11 the plasma contained 57 per cent of dye; on August 12, just before injection of more dye the reading

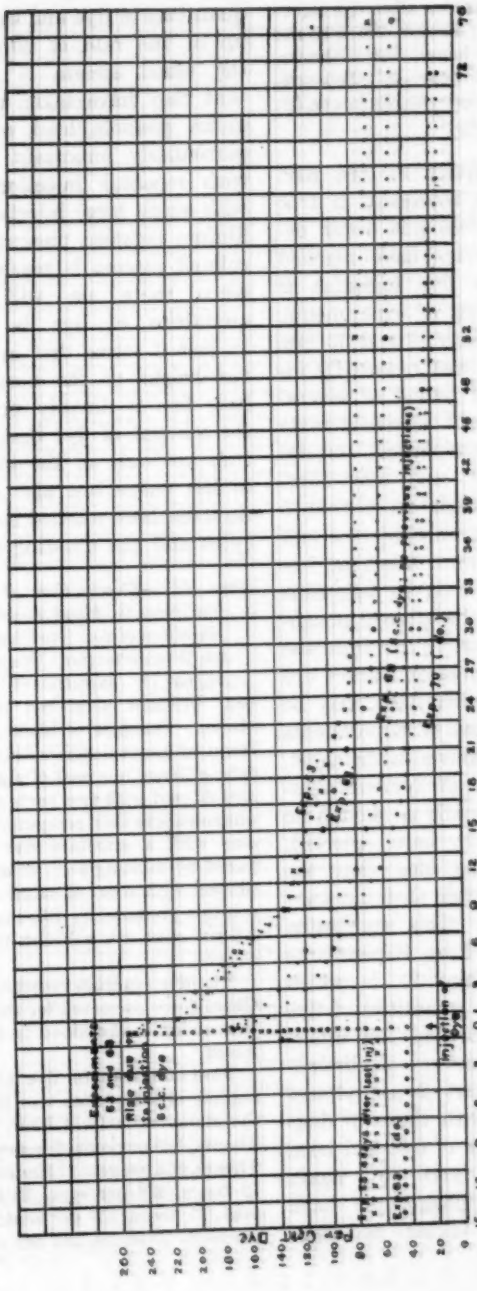

CHART 1

was 42 per cent; 4 minutes after dye injection, 249; 1 hour, 239; 3 hours, 206; 6 hours, 173; 9 hours, 135; 12 hours, 123; 15 hours, 105; 18 hours, 96; 21 hours, 87; 24 hours, 73; 27 hours, 67; 30 hours, 65; 52 hours, 52; 76 hours, 47; 100 hours, 47.

That this inactivity on the part of the phagocytes represents a true equilibrium and does not occur because the phagocytes have reached their capacity for dye ingestion is shown in a number of experiments. Thus, in Experiments 53 and 63 (see also Chart 1) dye was repeatedly injected and after a period of several days there was still a considerable amount of it in circulation, but its rate of elimination was very slow. At this stage of each experiment 5 cc. more of the dye were injected and samples of blood were taken at frequent intervals in order to determine how rapidly these dye-stained phagocytes could now remove dye from the plasma. The elimination curves are shown in chart 1 where they may be compared with those of Experiments 69 and 70. As is shown in the chart, the additional 5 cc. of dye was eliminated almost as rapidly as though no dye had previously been injected, thus disproving the idea that the phagocytes had reached their capacity for dye ingestion. This conclusion is further supported by Experiments 68 and 71 (see Chart 2), in which relatively enormous quantities of dye were injected. Allowing for differences in scale the curves of elimination shown in Chart 2 are almost identical in form with those in dogs to which small doses of dye had been given (Expts. 69 and 70), again proving that the phagocytes have the capacity for taking up large quantities of dye and that the slowing up of the rate of elimination from the blood stream is no indication that they have taken up their maximum possible load of dye. It is particularly important to note that with repeated injections of dye or with single large injections the equilibrium between phagocytes and dye-colored plasma is reached at a time when there are still considerable quantities of dye in the plasma. In fact it seems that in a rough way the height of the curve above the base line is directly proportional to the amount of dye injected. This is true not only of the initial portions of the curve but also after equilibrium has been reached between phagocytes and the dye-tinged plasma.

Expt. 68. Elimination of brilliant vital red from the blood stream after a large injection of dye. (see Chart 2).

Adult male terrier. Weight 20 kgm.

August 11. Injection of 35 cc. of 1.5 per cent brilliant vital red into the blood stream. Samples of blood were taken at frequent intervals thereafter and from each tube of blood one part of plasma was taken and diluted with two parts of saline. This unknown was then compared in a colorimeter with a standard dye solution prepared by diluting 5 cc. of normal dye-free plasma with 5 cc. of saline and 5 cc. of a solution prepared by diluting 0.75 cc. of the 1.5 per cent dye solution to 200 cc. with water.

The dye readings recorded below and in Chart 2 are corrected for the oxalate solution added to the blood tp prevent coagulation.

Four minutes after dye injection the dye reading of the plasma was 1032 per cent of the standard; after 1 hour, 946 per cent; 3 hours, 825 per cent; 6 hours, 680 per cent; 9 hours, 564 per cent; 12 hours, 484 per cent; 15 hours, 439 per cent; 18 hours, 392 per cent; 21 hours, 337 per cent; 24 hours, 320 per cent; 27 hours, 285 per cent; 30 hours,

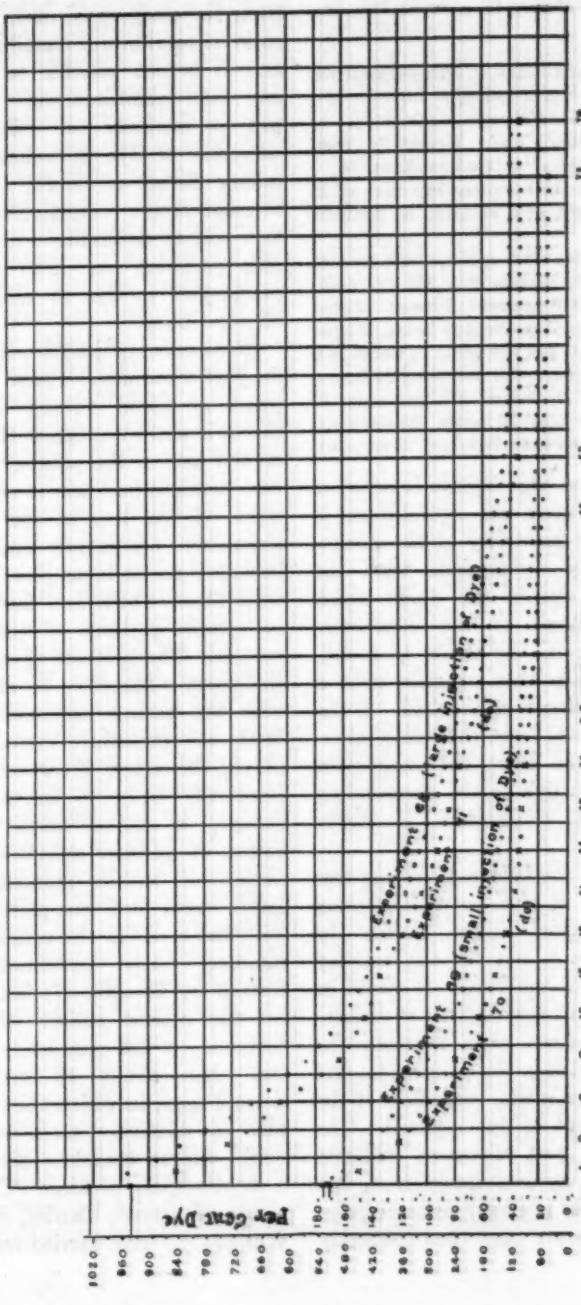
Per Cent Dye
Hours
Experiment of large injection of Dye
Experiment " " "
Experiment " small injection of Dye
" " "

CHART 2

262 per cent; 52 hours, 133 per cent; 76 hours, 111 per cent.

Expt. 71. Elimination of brilliant vital red from the blood stream after injection of a large amount of the dye (see Chart 2).

Male shepherd dog. Weight 16 kgm. Technique similar to that in Expt. 68.

August 12. Intravenous injection of 25 cc. of a 1.5 per cent solution of brilliant vital red.

Four minutes after dye injection the dye reading of the plasma was 945 per cent; after 1 hour, 840 per cent; 3 hours, 732 per cent; 6 hours, 621 per cent; 9 hours, 494 per cent; 12 hours, 444 per cent; 15 hours, 400 per cent; 18 hours, 358 per cent; 21 hours, 319 per cent; 24 hours, 274 per cent; 27 hours, 255 per cent; 30 hours, 235 per cent; 52 hours, 105 per cent; 76 hours, 98 per cent.

From these experiments it becomes clear that after an equilibrium is reached, the inactivity of the phagocytes simply corresponds with the amount of dye in the fluids which surround them. If the concentration of dye in the fluids is raised, phagocytosis is then resumed until a new equilibrium is established. If the dye is removed from the fluids (elimination by the liver), the phagocytes then give up dye to the surrounding fluids until equilibrium is again approached.

The exact conditions governing this distribution of dye between plasma and phagocytes might readily be studied were it not for the activity on the part of the liver. It would be interesting to know whether at equilibrium a constant ratio between the amounts of dye in the phagocytes and in the plasma exists, regardless of the actual amount of dye injected. The experiments just discussed indicate that this is approximately true, but since the liver is slowly removing dye from the plasma (and thus indirectly from the phagocytes also), the exact ratio at equilibrium remains unknown and it is not possible to formulate any rule quantitatively. With improved methods for following the excretion of dye in animals with a biliary fistula it should be possible to make proper correction for elimination and to determine with considerable precision the ratio of dye in phagocytes to dye in plasma under all conditions.

Elimination of dye from blood during the first 4 minutes. From the standpoint of blood volume determination it is well to recognize this activity on the part of the phagocytes; otherwise it might lead one to assume that much of the loss of dye from the circulation was merely apparent and in reality due to incomplete admixture with blood which had lain stagnant in certain portions of the vascular system. The best bit of evidence on this point is furnished by experiments with the carbon monoxide method. With the procedure commonly employed the escape of carbon monoxide from the body is impossible and samples of blood taken over the course of an hour should show diminution of carbon monoxide if the latter were not completely mixed with the blood in the course of a few minutes. Such experiments performed by Douglas (7) indicate that for the human subject the carbon monoxide is not completely mixed in less than about 15 minutes. In normal dogs, in which the circulatory rate is relatively more rapid, one might expect complete admixture in a shorter space of time. The experiments of Arnold, Carrier, Smith and Whipple (8) were carried out on dogs

over a period of about 16 minutes. No fall was observed between the sixth and sixteenth minutes. Recently numerous experiments have been carried out over an entire hour. In all cases there was a very slight fall after the sixth minute. On an average the carbon monoxide content of the blood at the end of an hour was 95 per cent of its value after 6 minutes. Samples taken at 30 minutes showed readings identical with those taken at the end of an hour. Centrifugalized samples of blood showed no change in the concentration of the blood to invalidate these figures. Such experiments might be assumed to indicate that admixture was not entirely complete in the 4 or 6 minutes allowed for admixture of the dye or carbon monoxide with the blood, although in normal dogs the error certainly is not more than about 5 per cent.

Despite the fact that experiments of this sort indicate that little error is made by assuming complete admixture with the blood in from 4 to 6 minutes, there still remains the possible criticism that within the interval of 4 minutes an extremely rapid elimination of dye from the circulation takes place. There is no reason to believe that the phagocytic activity would be enormously greater in the first minute or two than thereafter. The only positive evidence in favor of the idea is that furnished by some work of D. T. Harris (9). In his experiments samples of blood were taken at very short intervals following dye injection. He found that the dye concentration 30 seconds after the injection was much greater than it was several minutes later. From this he concluded that there was a rapid but transitory removal of the dye from the circulation within the first few seconds following the injection. This, however, is not the only interpretation possible. It seems much more likely that the high readings at the end of about one minute are the result of incomplete admixture of the dye with the blood. It is quite certain that the coronary circulation, for example, has a much shorter circulatory time than the extremities. Other parts of the body have rates of circulation peculiar to themselves. Indeed, if this were not the case, the dye would never become completely mixed with the blood, for it would circulate continuously with that particular sample of blood which happened to be in the heart at the time the dye was introduced. Samples taken thereafter would at one moment contain great quantities of dye and those taken several seconds later would contain no dye whatever. It is the inequality in length of the various circuits which breaks up this dye column and causes mixing of the dyed plasma with that which is not dye-tinged. It is probable that it takes several complete circuits of the blood before this admixture becomes anything like complete. Hence samples taken less than one or two minutes after the injection may be expected to show quite high figures at one moment and relatively low figures a moment later. It seems likely that this is responsible for some of the irregularities in the curves of samples obtained by Lamson and Rosenthal (10) one or two minutes after injection. This one might have expected but such experiments furnish no

grounds for criticising samples taken after a period of 4 minutes or later. Samples taken after the end of 4 minutes also showed some irregularities in their experiments, but this finding is quite contrary to the results of the numerous experiments of Dawson, Evans and Whipple (2).

Aside from these experiments of Harris there is one circumstance which does argue somewhat in favor of a rapid elimination during the first 4 minutes. This is the fact that the plasma volume figures obtained by the dye method are considerably higher than those obtained by either the carbon monoxide or the direct method of Welcker. It might be argued that this difference is the result of unrecognized elimination of dye from the circulation within the first 4 minutes following its injection. If these differences are to be accounted for in this manner, this unrecognized dye loss must be considerable, for whereas the dye method gives a plasma volume of 5 cc. per 100 grams of body-weight in a given animal, the carbon monoxide method or Welcker method gives a figure of only about 4.2 cc. (8). In the paper by Smith, Arnold and Whipple (8) the theory was advanced that this discrepancy is a result of uneven distribution of blood cells and plasma throughout the vascular system. It was thought that a certain amount of more or less stagnant plasma existed, perhaps in some of the smaller vessels, and that this plasma mixed readily with the dye and helped to dilute it, but was unable to dilute the carbon monoxide, since the latter is confined to the hemoglobin of the red blood cells. The evidence reviewed in that paper makes it seem likely that such a theory explains the difference in plasma volume obtained by the two methods, at least in part.

Of course it is conceivable that, as just stated, the difference between the two methods is partly or even entirely due to a rapid removal of the dye from the circulation during the first 4 minutes following injection. If such a process of elimination does occur during the first 4 minutes, it must be very transitory in nature. That this is necessarily so is shown in curves constructed from samples taken at frequent intervals during the first hour. Such curves are given in papers by Keith, Rowntree and Geraghty (1), by Hooper, Smith, Belt and Whipple (11) and by Dawson, Evans and Whipple (2). These observations have been repeated in connection with the experiments reported here, but since the results are quite similar to those reported by others the protocols will not be given in detail. Suffice it to say that an average of seven such experiments shows dye readings made against the usual standard of 82 per cent after 4 minutes; 79 per cent after 10 minutes; 77 after 20 minutes and 73 after 60 minutes. From these figures it is readily seen that during the first hour about 12 per cent of the dye is lost from the circulation. Calculation also shows that between the fourth and tenth minutes about 4 per cent is eliminated. This is 5 per cent of the total dye present at the time and, if figured on the basis of an hourly interval, would amount to 50 per cent of the total dye present per hour. Thus it is seen that between the fourth and tenth minutes the dye

concentration falls off at a rate about four times as rapidly as it does for the average of the entire hour period. But if the difference between dye and carbon monoxide methods is to be explained on the basis of a rapid elimination during the first four minutes it can be shown that the elimination during these few minutes must be far more rapid even than it is between the fourth and the tenth minutes following injection. Taking Experiment 69 with a dye reading of 177 per cent at the end of 4 minutes after dye injection, it is readily seen that, in order to obtain a plasma volume equal to that which would typically have been obtained with the carbon monoxide method, the dye reading would have to have been 211 instead of 177. The difference between these is 34 per cent, an amount which represents the amount of dye which would have to be eliminated in the first 4 minutes following injection. This is equivalent to an elimination in 4 minutes of 17.5 per cent of the total dye present, or figured on the basis of an hour, 263 per cent per hour. Thus, if the discrepancy between the two methods is to be explained by a rapid loss of dye during the first 4 minutes after injection, it is seen that the hourly rate of loss for that period would be 263 per cent, while the rate during the next 6 minutes is at a rate of only 50 per cent per hour. That phagocytes should be so active during the first minutes after injection and so much less active immediately thereafter would indeed seem remarkable in view of the fact that they are still far from being completely saturated with the dye. It remains a theoretical possibility that a small number of very unusual phagocytes might take up the dye at a very rapid rate at first and that within a period of 4 minutes they might reach their equilibrium with the dye-colored plasma. This seems to be a rather far-fetched conception. There also remains the possibility that such rapid and transitory removal of dye might be effected by a process of "adsorption" of it by certain of the tissues, but there is no evidence in favor of this idea, and microscopic examination of the tissue shows no obvious stores of adsorbed dye aside from small amounts taken up by elastic tissue, and even this is striking only when large injections of the dye have been made repeatedly.

Expt. 64. Dye and proteins in serum and lymph.

Female airedale. Weight 11 kgm.

July 24 to 30 inclusive: Daily injections of 20 cc. of 1.5 per cent brilliant vital red.

August 4. A sample of blood was drawn into a 15 cc. centrifuge tube containing 2 cc. of 1.6 per cent solution of sodium oxalate. After centrifugalization, the tube was found to contain 4.5 cc. of packed cells and 6.8 cc. of plasma including the 2 cc. of oxalate added to prevent clotting. After suitable dilution this plasma is read colorimetrically against a standard prepared by adding to 10 cc. of a mixture of saline with a small amount of normal dog plasma 5 cc. of a standard dye solution prepared by adding 0.75 cc. of 1.5 per cent brilliant vital red to 200 cc. of water. Comparison in the colorimeter shows that the plasma contains 294 per cent as much dye as the standard. Since the plasma was diluted with 2 cc. of oxalate solution the true dye concentration in the plasma would be 68/48 of this, or 416.

August 5. Another sample of plasma collected and read against a similar standard was found to contain 353 per cent dye after correction for oxalate solution had been made. The dog was then anesthetized with ether and lymphatics of various regions

were exposed. With a fine needle and a Record syringe it was easy to collect from the lymphatics small quantities of lymph. The entire operation took slightly more than an hour and at the end of this time the pulse, respiration and eye reflexes remained normal. A sample of blood taken at the end of the operation contained 5.2 cc. of cells and 7.2 cc. of plasma. This plasma was compared with the standard dye solution and, after correction for the oxalate solution, was found to contain 349 per cent of dye.

Analyses of the serum and of the lymph from which the fibrin had separated were made both for their dye concentration and for their content of protein. The proteins were determined by the method of Robertson (12) except that the albumin and globulin fractions were not separately determined. The non-proteins were estimated after precipitation with heat and acetic acid, as recommended by him, and the factor of 0.00200 taken as the refractive index of a 1 per cent solution of the combined albumin and globulin fractions. The results of the analyses are as follows:

Serum (taken immediately before operation).
Non-proteins 1.8 per cent.
Total proteins 6.2 per cent.

Serum (taken at end of operation)
Non-proteins 1.8 per cent.
Total proteins 6.2 per cent.

Liver lymph
Non-proteins 1.8 per cent.
Total proteins 5.5 per cent.
Dye concentration 218 per cent of above standard.

Cervical lymph
Non-proteins 1.3 per cent.
Total proteins 1.95 per cent.
Dye concentration 124 per cent of above standard.

Lymph from descending colon
Non-proteins 1.3 per cent.
Total proteins 1.75 per cent.
Dye concentration 88 per cent of standard.

Lymph from hind leg
Total proteins 1.9 per cent.
Dye concentration 88 per cent of standard dye solution.

Expt. 58. Dye and proteins in serum and lymph.

Male mongrel terrier. Weight 11 kgm.

August 13 to 17 inclusive. Daily injections of 20 cc of 1.5 per cent brilliant vital red.

August 24. Samples taken and dye and protein content of serum and lymph determined as in Expt. 64. The results of the analyses are as follows:

Plasma (taken immediately before operation) contains 326 per cent dye.

Plasma (taken at end of operation, 1 hour later) contains 340 per cent.

Serum (taken immediately before operation)
Non-proteins 1.9 per cent.
Total proteins 6.7 per cent.

Serum (taken at end of operation)
Non-proteins 1.9 per cent.
Total proteins 8.0 per cent.

Liver lymph
Non-proteins 0.8 per cent.
Total proteins 6.5 per cent.
Dye concentration 255 per cent of the above standard.

Cervical lymph
Non-proteins 1.0 per cent.
Total proteins 3.8 per cent.
Dye concentration 113 per cent of standard.

Lymph from hind leg
Total proteins 3.1 per cent.
Dye concentration 75 per cent of standard.

It would hardly seem, then, that the difference between the dye and carbon monoxide or Welcker methods could be explained by phagocytosis of the dye. The possibility still remains that the dye is eliminated from the blood stream not by phagocytosis or by adsorption but through diffusion of dye from the blood stream into the tissue fluids and lymph. This possibility has received little attention heretofore because no data existed upon which to base such a theory. As far as can be ascertained the

lymph has never been examined after injections of brilliant vital red. Experiment 64 gives the results of a trial of this sort. Large amounts of dye were given on successive days until a total of 2.1 grams had been injected. Five days were then allowed to elapse and at the end of that time the plasma was found to contain an amount of dye equal to 416 per cent when read against the standard used in ordinary blood volume determinations. On the next day another sample of plasma contained 353 per cent. It is evident that the phagocytes have practically reached an equilibrium with the dye-colored plasma and that the slow loss is due largely to dye elimination by the liver. On this same day samples of lymph were also examined. Lymph from the liver was found to contain 218 per cent of dye when read against the standard. Lymph from the cervical lymphatics contained 124 per cent; that from the descending colon 88 per cent; that from the hind leg also 88 per cent. Thus it is seen that the dye is most concentrated in the plasma. It is two-thirds as concentrated in the liver lymph; 35 per cent as concentrated in the cervical lymph; 25 per cent as concentrated in the lymph from the hind leg and 25 per cent as concentrated in the lymph from the colon. This gradually decreasing scale reminds one of the well-known fact that lymph contains less protein than serum and that the lymph from such regions as the hind leg contains less protein than that from the liver. This suggests the theory that the dye may reach the lymphatics by the same mechanism as the plasma proteins, for if so, they might be expected to occur in roughly the same ratios. To test this possibility the serum and lymph proteins were estimated and, just as in the case of the dye, the serum contained most; the lymph from the liver, neck, leg and intestine showing progressively less and less protein. The ratios are not exactly as in the case of the dye but the similarity in distribution of dye and proteins is rather striking. Such data do not offer conclusive evidence in favor of the mechanical origin of the lymph from blood plasma, but the parallelism between the behavior of dye and proteins is remarkable. In Experiment 58 similar results were obtained.

It was noted above that phagocytes sometimes abstract from the tissue fluids a large amount of their dye content. It is quite striking that lymph from the cervical lymphatics or from the lymphatics of the lower extremities may be almost entirely devoid of dye. It is only after long intervals when the phagocytes have had their fill of dye that much appears in lymph from these regions. In Experiment 55, for example, the lymph samples were collected about 10 hours after a large intravenous injection of dye. Since the phagocytes had not yet reached their equilibrium, the lymph from the cervical lymphatics contained only about 30 per cent of dye, whereas the plasma contained 281 per cent and the lymph from the liver contained 237 per cent. The presence of so much dye in the lymph from the liver may be due to the fact that the flow is so rapid that the phagocytes have not time to remove the dye.

Expt. 55. Dye and proteins in serum and lymph following recent injection of dye.
Mongrel male. Weight 18 kgm.

June 27, 11:50 p.m. Intravenous injection of 25 cc. of 1.5 per cent brilliant vital red.

June 28, 9 a.m. Ether anesthesia. A sample of blood was centrifuged and found to have plasma which read 353 when compared with a dye standard prepared as in Experiment 64. The serum contained 8.8 per cent protein. Lymph from the liver contained 218 per cent dye and 6.3 per cent protein. Lymph from the cervical lymphatics contained 2.6 per cent protein but only 30 per cent dye. Lymph from intestine and from hind leg was also examined, but the dye content was so small that accurate estimation was impossible. The content was obviously much below 50 per cent of dye.

Observations showing the passage of dyes and various colloids from the blood to the lymph are not lacking in the literature. Kuriyama (13) injected alkali blue into the blood stream and later found the substance in small quantities in the lymph. A number of investigators have found immune bodies in the lymph, but in most cases in quantities smaller than in the plasma. The literature on this subject has been reviewed by Osato (14) who also adds a number of his own observations on the same subject. He injected phenolphthalein into the blood stream and later found it in the lymph. The cervical lymph contained less of the dye than lymph from the thoracic duct. In his experiments bilateral nephrectomy was performed preliminary to the dye injection in order that the dye might remain longer in the blood stream. This precaution is not necessary when brilliant vital red is used, for the kidneys apparently play no part in the elimination of this dye from the body.

The relation which these observations bear to blood volume determination is at once evident. Since brilliant vital red mixes with the lymph as well as with the plasma it is important to know whether such passage of dye into the lymph takes place with sufficient rapidity to influence the blood volume determinations. Several experiments were performed to determine this point. Under light ether anesthesia an incision was made in the cervical region and preparations to collect lymph from the thoracic duct were made. Several cubic centimeters of dye were then injected into the blood stream. Within two or three minutes the dye appeared in the lymph flowing from the thoracic duct, showing that diffusion of the dye into the lymph must be quite rapid. It seems likely that the dye would have been seen in the lymph almost immediately if it were not for the fact that before the dye-tinged lymph can reach the cannula a considerable amount of dye-free lymph must first be displaced from the larger lymph vessels. In these experiments lymph collected after a lapse of 15 or 20 minutes contained between half and two-thirds as much dye as did the plasma collected at the same time.

We possess, then, conclusive evidence that some of the dye is promptly eliminated from the blood stream into the lymph. It seems quite likely that this accounts in part at least for the discrepancy between the dye and carbon monoxide methods. The high plasma volume in the case of the dye method is to be explained in part as due not so much to rapid removal of dye by phagocytosis or by "adsorption" as to a rapid transitory elim-

ination of dye into the lymph. The amount of dye thus lost from the lymph would rapidly become constant, for within a very short space of time the dye would be returning to the blood by way of the thoracic duct almost as rapidly as it was discharged into the lymph. It has already been observed that the carbon monoxide method gives a plasma volume of about 4.2 cc. per 100 grams of body-weight, whereas the dye method gives a value of about 5 cc. If the difference between the two is due *entirely* to tissue fluids and lymph which dilutes the dye but not the carbon monoxide, it is obvious that we shall have to postulate the existence of at least 0.8 cc. of these fluids per 100 grams of body-weight, and since the dye concentration in the lymph is only about 60 per cent of that in the plasma, the figure would be somewhat higher even than this, probably nearer 1.3 cc. of tissue fluid and lymph per 100 grams of body-weight. This seems to be a rather large amount of such fluids. On this basis there would be about 130 cc. of lymph in a 10-kilo dog, an amount equal to about 30 per cent of the known amount of plasma. Practically nothing is known concerning the amount of such lymph and tissue fluids in the body. There may be as much as this. If part of the discrepancy between dye and carbon monoxide methods is to be explained by uneven distribution of blood cells and plasma, with relatively stagnant quantities of plasma (8), or if in some unrecognized way part of the dye is lost from the circulation, then it will not be necessary to assume the existence of so much lymph and tissue fluid.

SUMMARY

A study is made of the behavior of brilliant vital red when introduced into the blood stream. The relations of these findings to the physiology of phagocytosis and to the theory of blood volume determination is discussed. It is found that this dye is slowly eliminated by the liver but not by the kidneys. Before being eliminated from the body much of the dye is taken up and stored by phagocytes. The phagocytes do not completely remove the dye from the plasma because the phagocytic reaction is a matter of equilibrium between phagocytes and the dye-colored plasma. The questions of loss of dye from circulation and the possibility of incomplete admixture of the dye and plasma are discussed in relation to blood volume determination. It is shown that dye passes in considerable amounts into the lymph and that, therefore, the blood volume figures include part of the lymph and tissue fluids also. This phenomenon doubtlessly explains in part the fact that the dye method gives figures which are larger than the carbon monoxide or direct method of Welcker. Other reasons for this difference are discussed.

In conclusion I wish to express my appreciation to Drs. Bloom, Kindell, Burky and Duncan for assistance in carrying out some of these experiments; to Mr. W. B. McNett for aid in making the charts, and to Dr. E. B. Carrier for proof-reading of the manuscript.

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Hemophilia

A Study of the Blood, the Clinical Course and the Heredity in Three Cases

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During the past two and half years we have had the opportunity of studying three cases of true hemophilia, two of these patients having been admitted to the hospital on several occasions for treatment for severe hemorrhage. Two of these men (E. H. T. and R. R.) belong to a single, large, typically hemophilic family, whereas the third has no family history of the disease whatever. The case records of two female members of the hemophilic family are also presented for comparative study.

Doubtless because of its peculiar hereditary nature and its striking clinical course, hemophilia was recognized as a distinct clinical entity at a very early date. That typical instances of the disease occurred in certain ancient Jewish families is quite evident from statements in the Talmud regarding the practice of circumcision in such families. The first description of unmistakable cases of hemophilia to appear in medical literature was that of Albucasis (1) early in the sixteenth century. It was not, however, until 1803 that wide medical attention was attracted to the disease by the paper of Dr. John C. Otto (2) of Philadelphia. In reporting typical cases in a certain family this writer gave a clear and concise description of its striking clinical nature, including an accurate statement of the hereditary law involved in its transmission from generation to generation. He concluded from his observations that males only are affected and that transmission occurs through unaffected females of the family only. Seventeen years later Nasse (3) formulated these same facts and, since that time, the now recognized law of heredity has gone by his name. Several monographs, reviewing reported cases and adding others to the list, then appeared in succession (4), (5), (6). Unfortunately, these, when taken together, tended to obscure rather than to clarify the hereditary picture of the disease in that they created a controversy regarding the questions of occasional transmission through the affected male and occasional occurrence of the disease itself in the female.

The most comprehensive and most reliable treatise on the hereditary phase of the subject now available is that of Bullock and Fildes (7), in which they critically analyze the records of all hemophilic families re-

ported in the literature up to the time of their publication in 1911. Regarding the questions raised by preceding writers, they conclude as follows: That no authentic case has ever been reported in which transmission of hemophilia has occurred through the male; that cases so reported were due to marriage of such male bleeders to females of hemophilic families, who were, therefore, the real transmitters of the disease; and that the few women described as bleeders were not true hemophiliacs but suffered from other hemorrhagic conditions. These authors could find authentic reports of but 44 hemophilic families in the literature. In spite of the convincing results of this investigation, one still not infrequently sees references in the literature to cases of hemophilia in females and to transmission of the disease by male bleeders. When critically investigated, however, such case reports are in our experience invariably unconvincing. It is in part for this reason that we wish to present our own cases which support Nasse's law.

The more recent papers devoted to hemophilia have dealt almost exclusively with the abnormalities of the blood. However, there is as yet no universally accepted explanation of the underlying etiology of the disease. Every known factor of importance in the normal process of coagulation has been claimed to be abnormal in hemophilia by some one of the many writers, who have offered theories regarding its etiology. Wright (8) believed that diminished blood calcium was a causative factor. Weil (9) concluded from his experiments that there must be an excess of anticoagulating material present. Morritz and Lossen (10), basing their ideas on the former's theory of blood coagulation, held the blood and tissue thrombokinase to be at fault. The careful studies of Addis (11) led him to attribute the abnormal prolongation of the coagulation time to a qualitative defect in the prothrombin, which manifested itself as a delay in the conversion of prothrombin to thrombin. Howell's (12) investigations convinced him that the prothrombin time, and hence the coagulation time, is delayed in hemophilia because of a quantitative rather than a qualitative deficiency in the prothrombin. More recently Minot and Lee (13) have advanced the theory of an hereditary platelet defect. Their work with hemophilic blood led them to believe that the qualitative or quantitative prothrombin defect observed by Addis and by Howell is due to abnormal resistance of the platelets. This, they believe, results in delay in the release of the contained prothrombin when hemorrhage occurs. They found the number of platelets in hemophilic blood to be normal. However, they further found that, when a concentrated suspension of such platelets was mixed with normal plasma containing added calcium, the coagulation time of the plasma was much longer than when a like suspension of normal platelets was added. They observed at the same time that a suspension of normal platelets added to hemophilic blood caused it to clot in practically normal time.

Without further discussion, it may be concluded from a survey of the most reliable recent investigations, especially those of Addis (11) Howell (12), Hurwitz and Lucas (14), Hess (15),

Laws and Cowie (16) and Minot and Lee (13), that a prothrombin deficiency alone is responsible for the prolonged coagulation time. It has been satisfactorily shown by the combined work of these investigators that the following factors are normal in hemophilia: blood calcium, fibrinogen, antithrombin, thrombokinase, serum proteins, number of blood platelets, blood hydrogen-ion concentration, bleeding time, capillary resistance and clot retractility and toughness. As will be seen from what follows, our own findings are in agreement with these.

METHODS

Throughout the work reported here a uniform technique for obtaining blood samples was employed. This consisted of removing blood, without unnecessary admixture with tissue fluids, from a superficial arm vein with a sterile Luer syringe previously rinsed with physiological saline solution. Howell's methods for determining the coagulation time and the prothrombin time were employed. Platelet counts were made by the method of Wright and Kinnicutt (17) according to which the normal count ranges between 200,000 and 400,000 per cubic millimeter. Serum calcium was determined by the method of Tisdall and Kramer (18). The capillary resistance test was performed according to the method described by Hess (15) and the bleeding time by the method of Duke (19). The quality of the platelets was studied by the procedure of Minot and Lee (13) with minor modifications. This consisted of separating the blood platelets from the oxalated plasma, so far as possible, by immediate centrifugalization, so that platelet suspensions in physiological saline and clear plasma from both normal and hemophilic blood could be set up together in various combinations. The optimal amount of 0.5 per cent calcium chloride, as determined by the procedure of Howell in his prothrombin-time method, was added to each sample of plasma after platelet suspension and clear plasma had been mixed. The platelet suspensions from the control and the hemophilic blood were made quantitatively as nearly alike as possible in each experiment.

CASE REPORTS

Case 1. R. R. (Hosp. No. 13277), white male, American, superintendent of automobile factory, married, aged 39 years. Admitted to hospital first on August 24, 1921, complaining of pain in left flank.

Family history. (See chart of family tree, Fig. 1, G. VI-12.)

Past history. The patient says that, because of frequent and severe hemorrhages he was practically an invalid during the first 13 years of his life, about 3 years of that time being spent in bed. At the time of puberty a marked lessening in the tendency to bleed was experienced. (He says that that this tendency to improve at the time of puberty has been observed in other bleeders of his family also). In 1911 he had a hemorrhage from the mouth for three weeks following the extraction of a tooth. Following the use of horse serum given for this, he had "joint disease" which incapacitated him for six months. In 1914, after an accident in which he received a severe blow over the left kidney, he had hematuria with severe pain over this region for three months. Off and on since that time there have been attacks of pain in the left flank, and occasionally on the right side as well, accompanied by severe hematuria, frequently traceable to injury.

Present illness. Pain in left flank prior to admission, similar to that of previous attacks. No gross hematuria observed by the patient during the present attack.

Physical examination. Moderately obese, healthy-appearing, middle-aged man. Occasional ecchymoses over skin surfaces. Heart and lungs normal. Abdomen slightly tender over right lower quadrant, but pain not reproduced by palpation over left flank. Genitalia normal. Neurological examination negative. X-ray studies of joints showed what appeared to be old arthritic changes. Kidney and x-ray plates revealed no evidence of calculi. Gastro-intestinal x-ray series essentially negative. Urine negative except for slight hematuria. Phenolsulphonephthalein test showed a total excretion of 66 per cent in two hours. Stools negative. Data from blood studies at various times are shown in the accompanying tables.

The family history of hemophilia and the personal history of frequent, severe hemorrhages, following slight injuries, together with the blood findings in this case, leave no doubt as to the correctness of the diagnosis. A point of special interest is that the tendency to prolonged hemorrhage was far more marked in the patient's early youth than later. No explanation for the improvement experienced by the patient at the time of puberty can be offered, but it is worthy of note that most of the other bleeders of the family are reported to have observed the same improvement after puberty.

Case 2. E. H. T. (Hosp. No. 15851) white male, aged 30 years, married, automobile factory worker, admitted first on December 22, 1921, complaining of hemorrhage from the gums.

Family history. Nephew to R. R. (See chart of family tree, Fig. 1, G. VII-25.)

Past history. Throughout his entire life the patient has suffered severely after any injury because of hematoma formation or hemorrhage at the site of an injury. Often laid up with joint swellings following bumps. In 1914 a slight injury to the left hip was followed almost immediately by swelling, stiffness and excruciating pain of this hip, which resulted in his being almost completely bed-fast for three months. Has always been subject to epistaxis of varying severity, attacks lasting from one to six days following slight trauma. In 1920 a slight accidental torsion of the left arm was followed by large hematomata from which he was again incapacitated for six weeks. Two years later he had a similar experience following a slight twist of the left leg.

Present illness. Began 11 days before admission with pain in the right upper jaw. Marked swelling and discoloration appeared first over the right side of face, the right eye bulging to a considerable degree. Swelling spread to neck and over upper part of chest. A large hematoma, which had formed in the roof of the mouth, ruptured three days prior to admission and had continued to bleed since that time.

Physical examination. Skin and mucous membranes very pale. The right side of hard palate was the site of a large hematoma from the center of which blood was slowly oozing from partially formed clots. Teeth showed evidence of much dentistry. There was proptosis and discoloration of the right eye. Pus was obtained from the right antrum. X-ray showed numerous periapical alveolar abscesses. From the site of the hematoma a considerable amount of pus was obtained on probing. Exposed bone was also detected. There was swelling and discoloration of the right side of the neck and down over the chest and abdomen nearly to the umbilicus. General physical examination otherwise negative.

Clinical course. In spite of local packing and the use of specially prepared dentures for stopping the hemorrhage, it continued two days longer. After seventeen days of local treatment for the sinusitis, during which time there was no more bleeding, the patient was discharged. Three weeks later, January 27, one of the infected molar teeth was extracted with hemorrhage lasting but one hour. One week after this, extraction of another tooth caused hemorrhage which lasted for six days, requiring readmission to the hospital. Because of objections from the patient's family, blood transfusion was withheld until the sixth day. By this time the hemoglobin had fallen to 24 per cent, the respirations had risen to 44 per minute and the pulse, almost imperceptible, had increased in rate to 160 per minute in spite of many other forms of treatment. After the patient had finally become extremely weak

and was delirious, permission for transfusion was obtained and 600 cc. of citrated blood were given intravenously. Bleeding ceased within 30 minutes after the transfusion was begun and the patient's condition showed remarkable improvement from this time on. There were no more hemorrhages during this admission, even when the packs were removed, and the patient was discharged two weeks later, the hemoglobin having risen to 43 per cent. Results of blood examinations at different intervals are recorded in Tables I and II.

This case and the succeeding one clearly indicate the seriousness with which infection and trauma must be regarded in the hemophiliac. It may be observed that hemorrhage was uncontrollable by ordinary measures, whereas blood transfusion acted specifically in checking it.

Case 3. C. J. (Hosp. No. 19,233), white school boy, aged 17 years. Admitted to hospital first on May, 1922, complaining of skin bruises.

Family history. (See chart of family tree, Fig. 3.) No history of bleeders in family.

Past history. Has always bruised very easily. Joints have always swollen and become painful after slight injuries. Left knee stiff since the age of five years, following a swelling which the family physician thought to be tuberculosis.

Present illness. Well developed boy. Slight swelling and stiffness of left knee and elbow joints due to hemarthrosis. Examination otherwise essentially negative. Urine normal. Wassermann test negative. Temperature normal. (Results of blood examinations are shown in Tables I and II.) Course showed gradual improvement without special treatment.

March 26, 1923. Readmitted, complaining of hemorrhage under the tongue following trauma with toothbrush. For several days had had pains in abdomen and swelling and pain in the right knee and left ankle.

Physical examination. A large hematoma under the tongue protruded into the submental space and into the mouth to such an extent as to prevent the jaws from closing. From a small abrasion over the sublingual surface blood was oozing slowly. Right knee and left ankle moderately swollen and tender, but not discolored. Physical examination otherwise negative. Urine negative.

Course. The patient was fed through a small esophageal tube and the mouth was frequently irrigated with a mild antiseptic solution. No further treatment was given. Bleeding stopped spontaneously on the second day after admission just before preparations for transfusion were completed. The hematoma gradually diminished in size and the patient was discharged on the ten day. When last seen he had had no recurrences of hemorrhage. (Results of blood examinations are given in Tables I and II.)

If we can rely on the family history, as given by the parents and other relatives of this patient, his case must be regarded as an example of sporadic hemophilia. Not the slightest evidence in favor of a family tendency to the disease was obtainable. And yet the clinical picture and the blood findings were entirely typical.

Case 4. A. M. T. (Hosp. No. 22,300), aged 26 years. Female of hemophilic family, sister of E. H. T., admitted to the hospital for tonsillectomy because of frequent attacks of sore throat.

Family history. (See chart of family tree, Fig. 1, G. VII, 27.)

Past history. Appendectomy in 1914 followed by no abnormal bleeding. Entirely normal menstruation. No history of unusual hemorrhage following injuries.

Present illness. Frequent attacks of sore throat during past two years.

Physical examination. Essentially negative except for evidence of chronic tonsillitis.

Clinical course. October 2, tonsillectomy under general anesthesia. There was no postoperative hemorrhage. (Results of blood examination shown in Table 1.)

While this patient, a potential transmitter of hemophilia, is the sister of E. H. T. and a niece of R. R., she has never shown any abnormal tendency to hemorrhage after

her various surgical operations, and her blood was found to be entirely normal.

Case 5. J. T. (Hosp. No. 14,314), single female, aged 19 years, niece of R. R. and cousin of E. H. T. First admitted October 6, 1921, complaining of periodic convulsions.

Family history. One brother died of hemorrhage. (See chart of family tree, Fig. 1, G-VII-23.)

Past history. Tonsillectomy and adenoidectomy. No postoperative hemorrhage. Normal menstruation since age of 11 years.

Present illness. Epileptic convulsions periodically since age of 5 years. Frequently bites tongue during attacks, but this is not followed by excessive bleeding.

Physical examination. Negative except for obesity and chronic bilateral maxillary sinusitis with nasal polypi.

TABLE I

Summary of results from blood examinations

CASE	DATE	COAGULATION TIME		PROTHROMBIN TIME		PLATELET COUNT PER CUBIC MILLIMETER	R.B.C. COUNT PER CUBIC MILLIMETER	W.B.C. COUNT PER CUBIC MILLIMETER	BLOOD Ca PER 100 cc.	HEMOGLOBIN
		Patient	Control	Patient	Control					
		minutes	*minutes*	*minutes*	*minutes*				*mgm.*	*per cent*
R. R.	2- 8-22	28	10	25	9	208,000	4,800,000	7,600		
	4- 3-22	56	14	37	12	219,000	3,888,000	6,350		56
	10- 4-22					242,000	5,060,000	7,050		100
	10- 5-22	33	12	26	11	252,000				
	10- 6-22					217,000				
	5- 2-23	70	17	40	9					
	3-30-23	53	15	48	13					
E. H. T.	1- 7-22	73	12	44	10		4,968,000	9,450	9.5	74
	2- 6-22	70				192,000	3,408,000	13,000		60
	2- 8-22	73	13	95	12	292,000				24
	2-19-22	43	15							43
	4- 2-23	44	14	72	13	220,000			10.5	
	5- 2-23	62	17	32	9					
	6-22-23	36	14	18	9	298,000	4,540,000			
	6-26-23	52	14	34	12	208,000	3,880,000			
C. J.	6-15-22					515,000	5,176,000	10,800		90
	6-17-22					289,000				
	3-27-23					260,000	5,160,000	7,600		110
	3-28-23	217	8	101	11	172,000			10.0	
	3-29-23					190,000	5,170,000			106
	3-30-23	172	15	163	13	234,000				
	3-31-23					320,000	5,800,000	11,000		116
	4- 2-23	387	14	166	15	182,000			9.6	
	4- 3-23					160,000	5,500,000			102
	4- 4-23	70								
	4- 5-23					270,000	5,750,000		9.1	100
A. M. T.	9-29-22	10	11	9	10	223,000	4,690,000			
J. T.	2- 8-22	14	11	8	8					
	4- 3-22	14	14	12	12	208,000		8,550		102

Clinical course. December 31, 1921, radical antrum operation without abnormal hemorrhage. February 28, 1922, operation for removal of nasal polypi without unusual bleeding. April 3, 1922, exenteration of ethmoid cells; not accompanied by post-operative hemorrhage. (Results of blood examination shown in Table 1.)

This patient, like the preceding, a potential transmitter of hemophilia, has shown no evidence whatever of the disease itself, having undergone several surgical operations without abnormal hemorrhage. Blood examination has revealed no abnormality, such as was found in the foregoing cases of hemophilia.

TABLE II

Effect of normal platelets on the coagulation time of recalcified hemophilic plasma and of hemophilic platelets on that of recalcified normal plasma

DATE	PLATELETS OF	PLASMA OF	COAGULATION TIME	REMARKS
			minutes	
March 30, 1923.....	Normal blood (C. C.)	Normal blood (C. C.)	13	Blood coagulation time of R. R., 53 minutes
	R. R. (patient No. 1)	R. R. (patient No. 1)	48	
	Normal blood (C. C.)	R. R. (patient No. 1)	15	
	R. R. (patient No. 1)	Normal blood (C. C.)	32	
	C. J. (patient No. 3)	C. J. (patient No. 3)	163	Blood coagulation time of C. J., 172 minutes
	Normal blood (C. C.)	C. J. (patient No. 3)	39	
	C. J. (patient No. 3)	Normal blood (C. C.)	45	
April 2, 1923.....	Normal blood (J. H.)	Normal blood (J. H.)	15	Blood coagulation time of E. H. T., 144 minutes
	E. H. T. (patient No. 2)	E. H. T. (patient No. 2)	136	
	Normal blood (J. H.)	E. H. T. (patient No. 2)	50	
	E. H. T. (patient No. 2)	Normal blood (J. H.)	40	
	C. J. (patient No. 3)	C. J. (patient No. 3)	265	Blood coagulation time of C. J., 387 minutes
	Normal blood (J. H.)	C. J. (patient No. 3)	52	
	C. J. (patient No. 3)	Normal blood (J. H.)	51	
May 2, 1923.....	Normal blood (E. C. D.)	Normal blood (E. C. D.)	12	Blood coagulation time of R. R., 70 minutes
	R. R. (patient No. 1)	R. R. (patient No. 1)	35	
	Normal blood (E. C. D.)	R. R. (patient No. 1)	14	
	R. R. (patient No. 1)	Normal blood (E. C. D.)	35	
	E. H. T. (patient No. 2)	E. H. T. (patient No. 2)	32	Blood coagulation time of E. H. T., 62 minutes
	Normal blood (E. C. D.)	E. H. T. (patient No. 2)	15	
	E. H. T. (patient No. 2)	Normal blood (E. C. D.)	28	

RESULTS OF BLOOD EXAMINATIONS

In Table I is presented a summary of results from the various blood studies made in the foregoing cases. It will be seen in regard to the first three

cases that the coagulation time of the blood and the prothrombin time were found on the occasion of every examination to be greatly prolonged as compared with the normal. In these same cases the platelet count, the blood calcium, the bleeding time, the nature of the clot and the capillary resistance test were all normal. The fourth and fifth cases, on the other hand, showed no abnormality of the blood whatever. The variability in the length of the coagulation time and the prothrombin time in the case of each hemophilic patient at different times is worthy of special note, but no cause for such variation in values was found.

The data presented in Table II show conclusively that the platelets of hemophilic blood are abnormal, as previously demonstrated by Minot and Lee. It is seen that a suspension of such platelets, when mixed with platelet-poor or platelet-free, recalcified, normal plasma, caused a very marked prolongation of the coagulation time, whereas a suspension of normal platelets had no such effect. Furthermore, when recalcified, hemophilic plasma was similarly treated with a suspension of normal platelets, its coagulation time was strikingly reduced.

COMMENT ON HEMOPHILIC FAMILY (CHART I)

The genealogy of this family has been traced through eight generations and dates back to the marriage of a Quaker woman, (G I-1) by the name of Jasper to Sir William Crispin in 1746. This union bore three children, two girls and one boy. The latter, (G II-3) bled to death from an accidental laceration of the scalp. The elder of the two girls (G II-1) did not marry, but her sister (G II-2) married and transmitted the disease to the third generation.

In the third generation there were two males and two females. While one of the males (G III-4) was apparently not a bleeder, the other (G III-3) suffered from frequent, severe hemorrhages and finally died from uncontrollable epistaxis. It is not definitely known whether one of the females (G III-1) married or not, but her sister (G III-2) transmitted the disease to the succeeding members of the family. Of the three sons of the latter (G III-2) two died in infancy and one lived to adult life without showing evidence of being hemophilic. The cause of the death in the cases of the two infants is not recorded, so that it is not clear whether they were bleeders or not. One of the two daughters in this family (G IV-3), though of a generation in which there were no proven bleeders, transmitted the disease. It has been impossible to trace the family record of her sister (G IV-1).

Seven children were borne by G IV-3, four males and three females. Three of the four males were proven hemophiliacs. One (G V-2) had frequent attacks of uncontrollable hemorrhage from the nose and teeth and was crippled during much of his lifetime by joint hemorrhages. His two sons (G VI-6, and G VI-7) and his grandson (G VII-5) were not sufferers from hemophilia. The second known bleeder of this generation (G V-3) died from uncontrollable epistaxis following a slight injury, while the third (G V-6) bled to death from a small laceration of the forehead. The

eldest male member of this family (G V-1) was apparently normal, as were all of his children and his grandchildren, no instance of prolonged hemorrhage ever having been observed.

Of the female members of the fifth generation one (G V-5) remained single. A second (G V-7) had three sons, G VI-10, G VI-11 and G VI-12) were proven bleeders. The remaining son (G VI-8) lived to the age of 50 and died of pernicious anemia, but never showed any tendency to hemorrhage. His children, two sons and three daughters showed no evidence whatever of hemophilia. The first of the

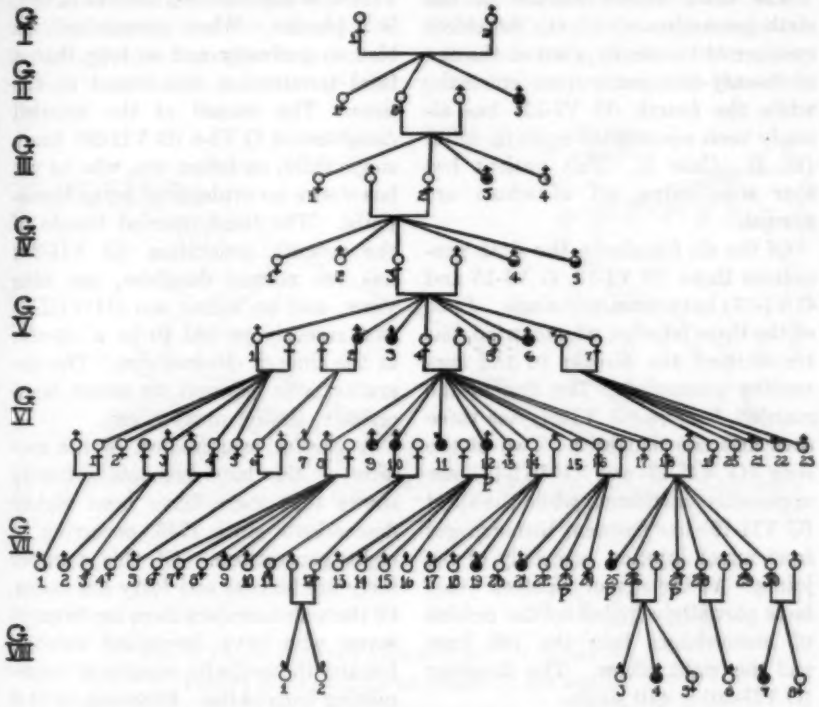

Fig. 1. Chart of Hemophilic Family Showing That Males Only Are Affected and That Transmission Occurs Through Unaffected Females

P = patient

none of whom were bleeders and two daughters, neither of whom have offspring. The remaining female of this generation (G V-4) passed the disease on to the sixth generation. She had eleven children, five sons and six daughters. Four of the sons (G VI-9, bleeders in the sixth generation (G VI-9) has had periodic attacks of hematuria all his life and superficial hemorrhages at frequent intervals from minor injuries. The second of the males manifesting the disease (G VI-10) now at the age of 46 years

has had many severe hemorrhages, entirely out of proportion to the relatively slight traumata suffered. Hemorrhages into the joints have been incapacitating at times. None of his four children, two sons and two daughters, has shown any tendency to hemophilia.

The third known bleeder of the sixth generation (G VI-11) the eldest member of his family, died at the age of twenty-one years from epistaxis, while the fourth (G VI-12) has already been commented upon in detail (R. R., Case 1). This patient has four sons living, all of whom are normal.

Of the six females in the sixth generation three (G VI-13, G VI-15 and G VI-17) have remained single. Each of the three females, who married, has transmitted the disease to the succeeding generation. The first of the married females (G VI-14) has three sons and one daughter. Two of the sons (G VII-17 and G VII-18) are apparently unaffected, while the third (G VII-19) has frequent hemorrhages from minor injuries, especially of the joints. At the age of nineteen years he is partially crippled by the residua of hemorrhages into the left knee and the right elbow. The daughter (G VII-20) is still single.

The second married female of the sixth generation (G VI-16) had one son (G VII-21) who died at the age of four years from hemorrhage after biting his tongue in a fall. None of his three sisters has shown any abnormal tendency to bleed. One of them (G VII-23) has been described in more detail elsewhere (J. T., Case 5).

The remaining female of the sixth generation (G VI-18) had five daughters and one son. The latter, a bleeder (G VII-25) has been discussed elsewhere (E. H. T., Case 2). Two of the daughters (G VII-29) and (G VII-27) (A. M. T., Case 4) are single. A third daughter (G VII-26) has twins nine years of age, a girl and a boy, who are normal. Her third child (G VIII-4) a boy eighteen months of age, is a bleeder. When circumcised, he bled so profusely and so long that a fatal termination was feared at the time. The second of the married daughters of G VI-8 (G VII-28) has a single child, an infant son, who as yet has shown no evidence of being hemophilic. The third married female of the seventh generation (G VII-30) has one normal daughter, age nine years, and an infant son (G VIII-7) who recently proved to be a bleeder at the time of circumcision. The operation was followed by severe hemorrhage, lasting many hours.

A careful examination of the records of this large hemophilic family shows that there have been eighty descendants since 1746, occurring in eight generations. Of this number forty are females and forty are males. Of the male members there are twenty-seven who have descended through females theoretically capable of transmitting hemophilia. Fourteen, or 51.8 per cent, are proven bleeders, while two who died in infancy may be classified as doubtful. The remaining eleven male descendants of the women who were potential transmitters were probably normal.

The thirteen male descendants of bleeders and potential bleeders in the family have never shown the slightest evidence of the disease. The female offspring of the male members of the

family have never transmitted the disease.

Of the forty female descendants in the family, twenty-nine or 72.5 per cent are potential transmitters of hemophilia. The remaining eleven or 27.5 per cent are descendants through the male line and will, therefore, not be considered further, since none of them has transmitted the disease. Of the twenty-nine through whom transmission might occur, seventeen or 58.6 per cent have remained single. Ten, or 83.5 per cent, of the twelve who married have actually transmitted the disease. The remaining two have not, so far as is known, transmitted the disease to their sons. Of course in the case of G VII-28 it is not justifiable to draw conclusions as yet, since her only son is but eighteen months of age and has never been circumcised or subjected to other traumata that might bring to light the tendency to abnormal hemorrhage.

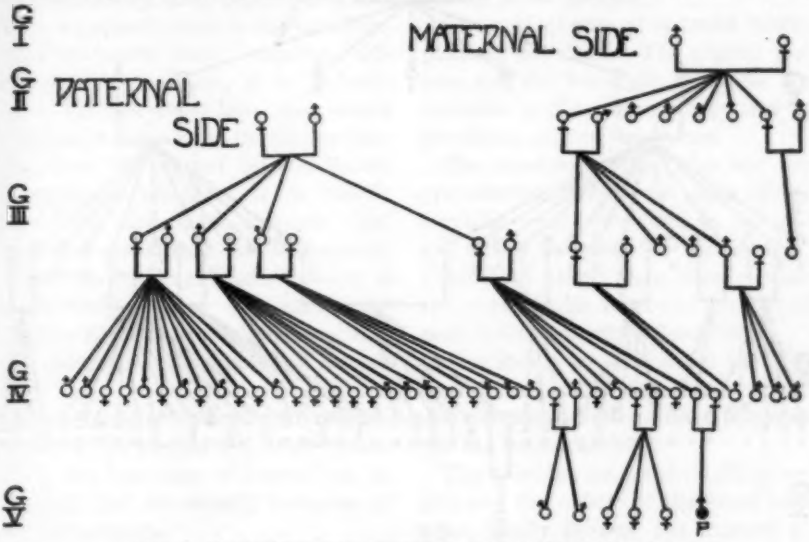

FIG. 2. CHART SHOWING ANCESTRY OF C. J. (CASE NO. 3)
Patient is the only known bleeder in this family

COMMENT ON CHART II

The accompanying chart of the known ancestry of C. J., Fig. 2, is presented as evidence of the sporadic occurrence of true hemophilia. On the maternal side there were ten males who would have been subject to hemophilia, according to Nasse's law; yet, none of these and none of the other members of either family ever showed the slightest evidence of the disease.

DISCUSSION

The important points to be emphasized in a consideration of hemophilia are those pertaining to the blood abnormality found and those dealing with its peculiar hereditary character. The data presented here from repeated blood studies in three cases of

the disease are of special interest, in that they confirm the findings of Howell, regarding the deficiency of prothrombin, and at the same time those of Minot and Lee regarding a defect in the blood platelets. If it be assumed that the platelets are the source of prothrombin in the shed blood, there appears to be no incompatibility between these two views. It is quite clear that the observed platelet abnormality, manifesting itself as a delay in their disintegration, accounts for the apparent quantitative deficiency demonstrated.

Two facts regarding heredity in hemophilia are clearly illustrated by the case records presented. The first is that sporadic cases do apparently occur, since no family history of the disease whatever was obtainable in Case 3. The second point exemplified is that Nasse's law accurately depicts the hereditary features characteristic of the disease. Before the diagnosis of hemophilia can be accepted for those cases of hemorrhagic disease with a hereditary history at variance with the law of Nasse, it is necessary that the characteristic blood changes be demonstrated. Thus far, no such corroborative evidence has been brought forward to support the claims of those who insist that hemophilia occasionally occurs in females and is transmitted through males.

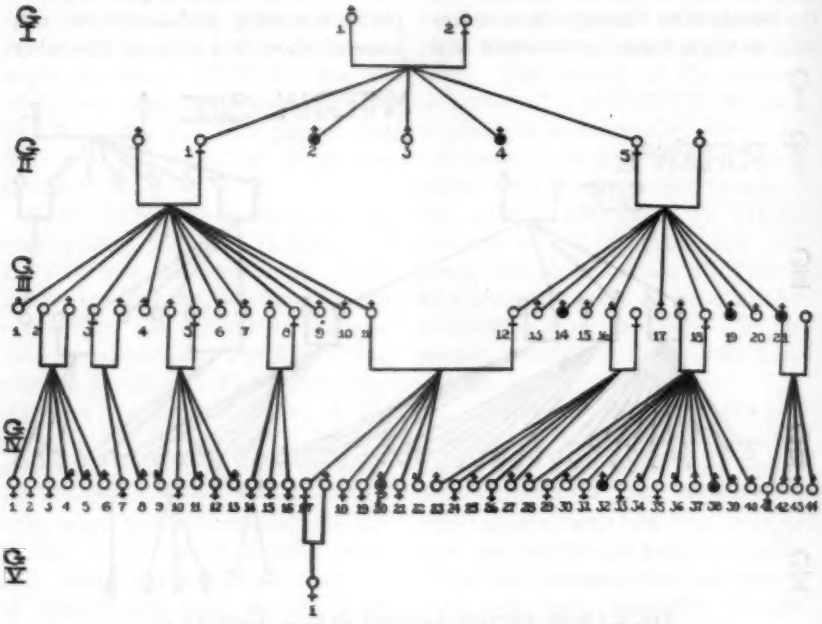

FIG. 3. CHART OF HEMOPHILIC FAMILY WITH INTERMARRIAGE OF COUSINS

Hemophilia was transmitted to the patient through the mother, according to Nasse's law, and not through the father. (See under Discussion.)

In Fig. 3 is shown a chart of a large hemophilic family of Russian Jews in which there was intermarriage of cousins. The young son (G IV-20) of this couple was found by us to be a true hemophiliac. The case is presented to illustrate a common error in the interpretation of family histories, which results in erroneous conclusions regarding the transmission of hemophilia. The two different branches of the hemophilic family represented by these parents had long been separated prior to the marriage. Had the history from the father's side alone been available, it is probable that this case would have been looked upon as an example of the disease having been transmitted by the father, a potential bleeder. It is readily seen from the chart, however, that such an assumption is unnecessary, when the maternal family history is considered, because this shows the mother to be capable of transmitting the disease in accordance with Nasse's law.

The cases of hemorrhagic disease occurring in women are in all probability not true cases of hemophilia, as claimed, but are usually instances of chronic purpura.

SUMMARY AND CONCLUSIONS

A typical, large, hemophilic family is described in which 14 proven cases of hemophilia have occurred in eight generations. Detailed reports of two of the cases representing the sixth and seventh generations are presented. Two normal female members of the same family presented no evidence of the disease, either clinically or from blood examination. The hereditary transmission occurred in this family according to the law of Nasse. No occurrence of the disease in females and no transmission through the males of the family has been observed. Of the females capable of transmitting the disease 83.3 per cent are known to have done so. Among the potential hemophiliacs 51.8 per cent have proved to be bleeders.

One typical case of sporadic hemophilia is described. The clinical picture and the blood abnormalities are identical in the sporadic type and in hereditary type of the disease.

The blood coagulation time and the prothrombin time in the cases of hemophilia were from twice to twenty-five times as great as the normal. They both varied from time to time to a considerable degree in the case of each individual with hemophilia.

The finding by Minot and Lee of a qualitative platelet deficiency without a diminution in the number of platelets has been confirmed.

The bleeding time, the capillary resistance, the nature of the blood clot when finally formed, the amount of blood calcium and the number of blood platelets were at all times found to be normal in the cases of hemophilia studied.

Blood transfusion is the most effective form of treatment for the hemorrhage in hemophilia and should be employed as a prophylactic measure preceding surgical operations.

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The Excretion of Guanidine Bases in Two Cases of Arterial Hypertension with Reduction in Blood Pressure

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PATIENTS with arterial hypertension, who are admitted to the hospital with marked myocardial insufficiency and edema, often have a rapid fall of blood pressure following treatment. This fall in blood pressure is often particularly striking when preceded by a marked diuresis. This observation suggests, among other things, the possibility that an increased diuresis eliminates an excess of some pressor substance which has accumulated in the patient's body.

In several previous articles (1, 2, 3) attention has been called to the possible rôle of guanidine compounds in the production of arterial hypertension. In two patients recently seen in this clinic, we have studied the daily output of guanidine bases during the period in which a marked fall in blood pressure took place. These patients received digitalis and diuretin, and there was an increased diuresis accompanying the fall in blood pressure.

The guanidine determinations were made according to the method of Findlay and Sharpe. The crystals obtained have had the correct nitrogen content (26 per cent) and the correct melting point (225°C.) for dimethyl guanidine picrate. A mixture of these crystals with known crystals of dimethyl guanidine picrate gave a sharp and uniform melting point.

This method does not convert creatine or creatinine into dimethyl guanidine picrate. The urine from these patients did not contain creatine when tested by Folin's method. The addition of 10 grams of creatine or of 10 grams of creatinine to 1 liter of urine produced no change in the content of dimethyl guanidine picrate when compared with another liter of the same urine.

The results of the observations on these two patients are shown graphically in Figs. 1 and 2. It will be seen that the fall in blood pressure was accompanied by a gradual rise in the output of dimethyl guanidine. The output for several days after the fall of blood pressure was quite high, suggesting the previous retention of an excess in the body. In both patients there was an increase in the urinary output, the increase in one case being quite striking.

The above observations suggest a relationship between the guanidine

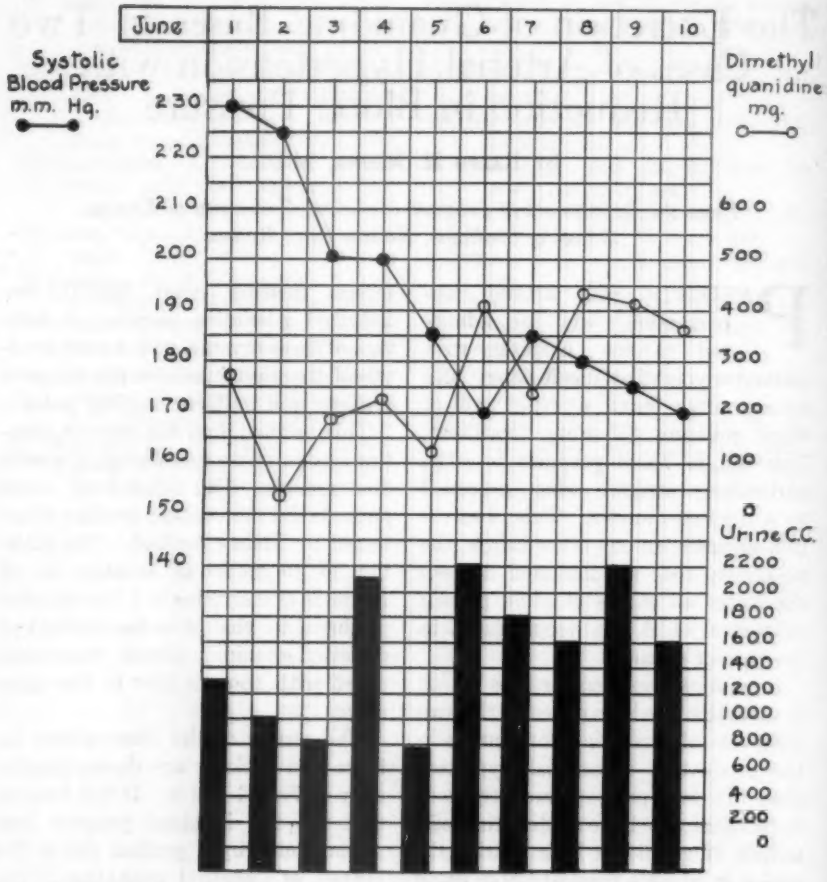

FIG. 1. CHART SHOWING FALL IN BLOOD PRESSURE ACCOMPANYING INCREASED OUTPUT OF DIMETHYL GUANIDINE

Patient a white man, aged 52. Marked cardiac enlargement, enlarged liver, edema and ascites. Blood urea on June 1, 13 mg. per 100 cc., on June 12, 10 mg. per 100 cc. Phenolsulphonephthalein test, 50 per cent on June 12.

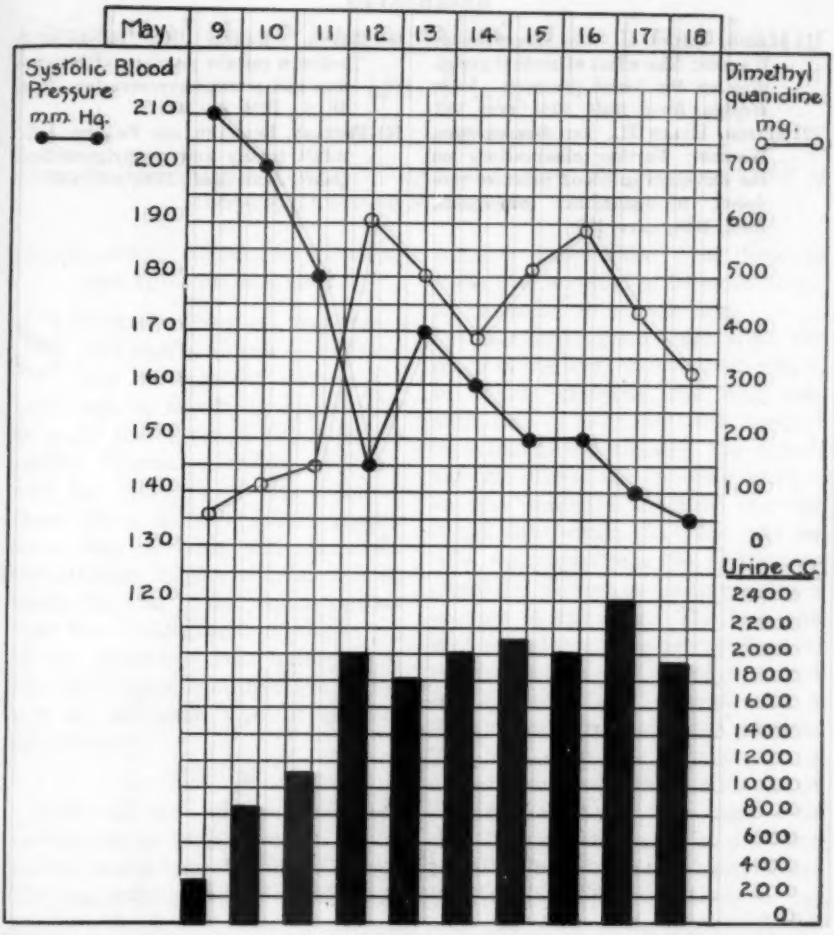

FIG. 2. CHART SHOWING FALL IN BLOOD PRESSURE ACCOMPANYING INCREASED OUTPUT OF DIMETHYL GUANIDINE

Note marked diuresis. Patient a negro woman, aged 41. Heart moderately enlarged, slight edema of feet. Blood urea on May 19, 6 mg. per 100 cc.

bases and hypertension, since the marked increase in excretion of these bases was followed by a fall in blood pressure.

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Origin of the Phagocytic Cells of the Lung of the Frog

By Margaret R. Lewis

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ORIGIN OF THE PHAGOCYTIC CELLS OF THE LUNG OF THE FROG

SINCE the lung of amphibians and reptiles is transparent and can be distended outside the body wall, it affords an opportunity to study the ingestion of particulate matter *in vivo*. Although undoubtedly the structure of this organ in these lower animals differs greatly from that of birds and mammals, nevertheless, in general plan it is so much like that of the higher animals that the establishment of the origin of the phagocytic cells formed in it may throw some light upon the formation of the same type of cells in mammals.

METHOD

Most of the observations were carried out on frogs, some on toads, snakes, and a large California lizard. The lung of the snake and of the lizard are more simple in structure than that of the frog and correspondingly clearer. The results, however, were the same.

For short observations the frog was anesthetized with ether vapor; for long observations it was first placed in a weak solution of chloretone in water until it became quiet, and later a few drops of weak chloretone in salt solution were added from time to time, over a period of 24 to 48 hours, to the moist cotton covering.

When the frog was quiet, a slit was made in the body wall through which, by gentle pressure, the lung was extruded. A tube was then inserted into the glottis through the mouth and air blown into the lung until it became distended to about the size it had been within the body. As the tube was withdrawn the glottis was closed by ligature so as to prevent a collapse of the lung. The frog was then placed in a large petri dish under the microscope and all of it except the distended lung covered with a layer of cotton wet with salt solution. The lung itself was kept moist by dropping salt solution over it. Most of the observations were made with the 16 mm. lens and No. 18 Zeiss ocular. For more careful study of the cells, a coverslip was placed on the lung and observations made with the 2 mm. lens and No. 6 ocular.

After the circulation had been studied for ten to twenty minutes, to determine that there were no abnormalities due to trauma, the heart was exposed and a small quantity of suspension of India ink (Higgin's waterproof India ink) introduced into

the circulation by way of the heart. This suspension was prepared, just before being used, by adding one drop of ink to 5 cc. of distilled water—in some instances salt solution—and filtering it twice. This method gave an even suspension composed of minute carbon particles less than one micron in diameter, which, when examined fifteen minutes later, was found to be unchanged, regardless of whether it had been prepared in water or in salt solution; later some agglutination did take place in the suspension prepared in salt solution.

The amount of ink suspension introduced into the circulation depended upon the size of the frog; in the larger ones usually about one drop, in the smaller ones half this amount.

The salt solution found to be best suited for use in these observations was a diluted Locke solution from which the sodium bicarbonate had been omitted—67 cc. of Locke solution and 33 cc. of distilled water.

OBSERVATIONS

When the lung was observed under the microscope, it appeared as a clear bag divided into smaller compartments by short septa, which extended toward the inner surface of the lung and in which were the large blood vessels. The inner walls of these chambers were composed of epithelial cells over which was spread a network of small capillaries, giving the appearance of channels of blood flowing around islands of epithelial cells, each island being composed of from 4 to 8 or more epithelial cells and their size depending upon the amount of distention in the lung.

The capillary wall was composed of a single layer of endothelial cells. Over the capillaries were scattered a number of smooth-muscle fibers and the whole covered over with a layer of large, more or less hexagonal cells—the pleura. Both the pleura and the smooth-muscle cells were practically transparent and could not be distinguished while the preparation was alive. Most of the epithelial cells lining the spaces were ciliated. The layer of tissue over the surface was so thin that when the glottis was closed aeration readily took place from the outside air. If, therefore, the lung was kept moist with salt solution, the circulation remained normal, the cilia continued to beat, and some contraction of the smooth muscles was exhibited, sometimes for two or three days, provided the frog was kept cold.

One marked difference between the lung of the amphibian or the reptile and that of birds and mammals is the total absence of dust cells and the small number of clasmatocytes usually present. The clasmatocytes were large and all of those observed contained some ingested material, either melanin granules, red blood-cells, yellow-green masses, or brown-red crystals.[1] This type of clasmatocyte is not peculiar to the lungs; apparently all of the clasmatocytes of the frog, whether located in the lungs, liver, spleen, bone-marrow, lymph nodes, or subcutaneous tissue, contain more or less of this ingested material. Most of the clasmatocytes were not seen in the distended lung, as they

[1] Dr. A. R. Rich, who tested this material, found that the brown-red crystals were bilirubin and the yellow and yellow-green substance was ingested red blood-cells in various stages of digestion.

lay toward the inner surface, usually just above the ciliated epithelial cells covering the deeper septa, and often with processes protruding out between the cilia in the lung cavity. These were observed when the lung was cut up in salt solution containing neutral red.

The reptile and amphibian, also some fish, differ from the higher forms in that there is present in the blood-stream an occasional pigmented leucocyte. This cell is about the size of the mononuclear leucocyte and contains melanin granules. There may be only a few isolated melanin granules in a leucocyte, or there may be several masses of them, almost completely filling the cytoplasm of the cell. These cells may be readily differentiated from cells carrying carbon by the size, shape and color of the pigmented granules, which are large and of even contour, either round or rod-shaped, and of a dark brown color, while the carbon particles are irregular black clumps of varying sizes.

In the lung of the uninjected frog the blood courses swiftly through the capillaries, and while the white blood-cells are often on the outer edge of the current they do not linger along the walls but pass rapidly through the capillaries. The lumen is little wider than a red blood-cell, so these cells usually go through one by one and twist and bend as they are carried around the corners of the epithelial islands. The circulation in these uninjected frogs remained vigorous and no white blood-cells were observed to lodge in the capillaries, even when kept under observation for as long as four hours.

After the lung had been studied, the suspension of carbon, usually less than one drop, was slowly introduced into the heart in two doses, three or four heart-beats apart.

One of the surprising features of these observations, a fact which has not heretofore been taken into account by investigators, is that the carbon, as it came in contact with the colloidal solution of the blood, began to agglutinate; so that, instead of a fine suspension of microscopic particles, there were formed in the blood-stream much larger particles and some threads of different sizes (Figs. 1, 2 and 3), which stuck to the white blood-cells and occasionally to the endothelial cells, not only in the capillaries but also in the larger vessels. Some of the delicate threads of clotted suspension were caught at one end and waved back and forth in the blood-stream; others were attached more nearly the middle to one of the angles of the epithelial islands, in which case the two ends undulated. Such a thin, delicate thread of carbon often became plastered against a capillary wall, owing to the rush of red blood-cells past it; again, one or two leucocytes became entangled with it and stopped there. The smaller clumps of carbon passed along in the blood-stream, where they soon became stuck to white blood-cells, for the most part large mononuclears, but there were also some lymphocytes and even an occasional polymorphonuclear cell with a few particles of carbon.

The behavior of the carbon depended largely upon the amount of ink in the suspension and the amount of suspension injected. If the suspension was too heavy, it remained for some time in the heart and there

formed into particles of such size that, when they got into the lungs, they formed emboli. If a large quantity of dilute suspension was injected at once, the carbon particles became deposited along some of the endothelial cells and thus obscured the picture. The greatest number of phagocytic cells could be produced by injecting a small quantity of a weak suspension in two or three doses, all within one minute. In this way most of the carbon became agglutinated on the leucocytes, which soon lodged along the capillary bed of the lung.

After the carbon had become attached to a leucocyte the latter no longer moved with the blood-stream, but lingered along the wall of the blood vessel, in some instances to be loosened again and carried farther, possibly to the next angle of the capillary bed, where it became lodged. In the course of this slow progress several white blood-cells often became massed together and more carbon particles entangled with them, so that clumps of two or three leucocytes with carbon particles, as well as individual cells carrying carbon, lodged along the vessel walls. The red blood-cells rushed through a capillary in which leucocytes and carbon were stuck, but occasionally the mass, augmented by an entangled red blood-cell, was large enough to block the passage entirely, in which case the circulating blood took a different channel. Emboli were also formed of carbon particles alone, for while at first none of the carbon particles were large enough to block a capillary, some of the larger ones did sometimes become stuck together into masses which formed emboli. Into such a capillary slipped an occasional leucocyte, until in places the embolus was surrounded by leucocytes. All of this occurred within a surprisingly short period of time. Often within two or three minutes after the carbon had been injected into the heart none of it was to be seen circulating in the blood-stream, while particles were either sticking to leucocytes or to the walls of the blood vessels.

FIG. 3. Drawing (× 530) from the lung of another frog in which the liver and spleen had been ligatured before the carbon was introduced. The blood passed over the lodged leucocytes, three of which contained carbon and the fourth, in the upper right corner, had a thread of carbon wrapped around it. The drawing was made fifteen minutes after the carbon was introduced.

FIGS. 4, 5 and 6. Drawings (× 1500) of white blood-cells made ten minutes after a minute amount of suspension of carbon had been added to freshly drawn blood in a hanging-drop preparation. Figure 4 shows six leucocytes entangled in a mass of carbon. Three of these cells contain carbon granules. This corresponds to the formation of an embolus. Figure 5 shows a mononuclear leucocyte containing carbon and a clump of carbon sticking to it. Figure 6 shows a smaller mononuclear leucocyte containing carbon.

FIG. 7. Drawing (× 1500) from the same lung as that of Figure 3 made one hour later. It shows some of the large carbon-laden cells lodged in a large blood vessel. The carbon was in the form of clumps partly composed of nonpigmented substance. When neutral red was placed on these cells almost no red appeared in the cells.

FIGS. 8, 9 AND 10. (× 1500.) Cells in the same hanging-drop preparation as those of Figures 4, 5 and 6, drawn 24 hours later. The cells shown in Figures 8 and 10 were hypertrophied and their nuclei had lost their characteristic leucocytic appearance.

All drawings were made with the camera lucida.

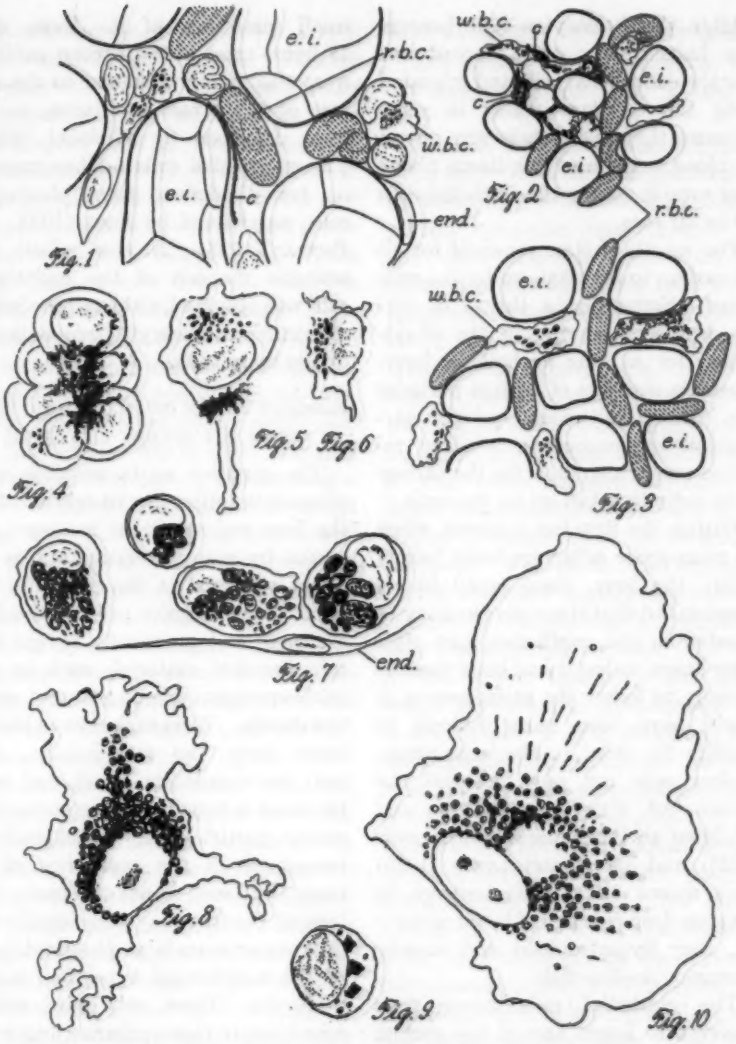

FIG. 1. Drawing (× 530) showing lung of a frog following injection of carbon into the heart. A thread of carbon was stuck to a corner of an epithelial island (*e.i.*) and three white blood cells containing carbon were lodged in the capillaries. At the end of two hours the white blood cells were in the same region but the thread of carbon had become entangled with a leucocyte and been carried away.

FIG. 2. Drawing (× 530) from a lung showing an embolus formed by two clumps of carbon, two leucocytes and three carbon-laden cells. At the upper right side is lodged another carbon-containing cell. The blood passed over this cell and also over the one forming the lower left corner of the embolus. Both the spleen and liver were tied off before the carbon was introduced into the heart. The drawing was made ten minutes later.

(*See opposite page*)

After the leucocytes had become thus lodged they often spread out over the endothelial cells and migrated along the capillary walls; in some instances they made their way out of the blood vessel into the tissue above it, or even in among the epithelial cells of the air sacs.

The length of time required for all the carbon to be taken up by the cells varied. Sometimes a thread of carbon was seen waving in the blood-stream for as long as half an hour. When an embolus of carbon particles was formed, although quickly surrounded by leucocytes, it often required one or two days for the carbon to be entirely taken up by the cells.

Within the first few minutes, when the phagocytic cells were being lodged within the lung, there could be no question but that these were leucocytes arrested in the capillaries, but after a few hours, unless it had been possible actually to follow the phenomenon, it would have been more difficult to identify the cells, for the large mononuclear cells not only ingested the carbon but divided by mitosis and exhibited all the changes which Lewis (1925) and Lewis and Lewis (1925) have shown these cells undergo in hanging-drop cultures of blood alone—i.e., they hypertrophied and became clasmatocyte-like cells.

The epithelial cells never were observed to ingest any of the carbon and the air sacs remained intact. The endothelial cells also remained unchanged and were not observed to become macrophages. Even in the experiments in which too large a quantity of suspension was injected, so that it became plastered along the endothelial walls, these cells ingested only small quantities of it. Even after 48 hours most of the carbon particles were still merely adherent to the surface of the intact capillaries, as has been described by Wislocki (1924). The endothelial cells neither rounded up nor divided to form phagocytic cells, as claimed by Foot (1923) and Permar (1923). Neither mitotic nor amitotic division of the endothelial cells was observed, although the lodged mononuclear leucocytes frequently did divide by mitosis.

POSSIBLE ORIGIN OF PHAGOCYTIC CELLS FROM THE SPLEEN AND LIVER

The question as to whether these phagocytic cells may be released from the liver and spleen, as has been suggested by many investigators, is one easily answered in the frog. In this animal, the Kupffer cells of the liver are large and practically always contain ingested material, such as bilirubin crystals, melanin granules, or red blood-cells. Were such cells to become loose from the endothelium, drop into the circulating blood, and reach the heart in time to take up some of the carbon particles, they could easily be recognized in the capillaries of the lungs; but such is not the case. The lung of the frog may occasionally contain one or more large phagocytic cells of the Kupffer-cell type, i.e., a clasmatocyte. These cells were seldom seen lying in the capillaries, but where they did they, also, took up some of the carbon particles, provided such particles came in contact with them. There was no increase in the number of these clasmatocytes following the injection of carbon into the blood-stream and the many carbon-laden cells that accumulated in the lungs

did not contain ingested red blood-cells, melanin granules, or bilirubin crystals—they were unquestionably leucocytes.[2] They did not arise from the Kupffer cells by division, because it requires from thirty minutes to an hour or more for a cell to divide and the phagocytic cells were formed in the lungs in a much shorter time. That they did not arise from the spleen is partly answered by the same arguments, for many of the cells of the spleen of the frog also contain ingested melanin granules and red blood-cells. There are, however, many small cells in the spleen which lack ingested material and whether or not the phagocytic cells had their immediate origin from these could only be determined by tying off the spleen before the carbon was injected. Such an experiment was carried out on four frogs, without in any instance obtaining a decrease of the macrophages in the lung. In three other frogs both the liver and spleen were tied off just before the carbon was injected into the heart, and in all of these the carbon became attached to leucocytes and the leucocytes became lodged in the capillaries of the lung. Within five minutes after the carbon was injected the lungs appeared to contain as many cells with ingested carbon (Figs. 2 and 3) as in the experiments in which the liver and spleen were not ligated and one hour later there were many typical macrophages even in the larger vessels (Fig. 7). Foot and Wislocki have both shown that the removal of the spleen from rabbits before carbon is introduced does not cause a decrease in the number of phagocytes in the lungs.

[2] Throughout this paper *leucocyte* is used in the sense of white blood corpuscle.

ACCUMULATION OF MACROPHAGES IN THE LUNGS AFTER INJECTION OF CARBON INTO THE LYMPH

A simple method of obtaining phagocytic cells in the lungs of the frog was by injecting ½ cc. of the suspension of carbon into the dorsal lymph sac. The time elapsing before the first appearance of ink-laden cells in the lungs varied in different frogs, but in all of the ten so treated there were many such cells present within an hour. They accumulated slowly and the carbon was not all lodged in the lungs within the first few minutes, as it was when injected through the heart, but cells containing carbon continued to arrive there for as long as 48 hours. The blood from the heart during this time contained a few free clumps of carbon and a number of cells with ingested carbon.

The lymph found in the dorsal lymph sac varied in quantity and also in the number of cells present in it in each normal frog examined. In three frogs it was scanty and contained only a few small mononuclear cells. In two others it was possible to obtain several drops of lymph and prepare hanging-drop cultures from it. This lymph contained a number of lymphocytes, some large mononuclear leucocytes, a few clasmatocytes (with ingested red blood-cells and melanin granules), and one pigmented leucocyte. Two cells in mitotic division were found, but no great increase in the number of cells took place in the cultures. Some of the injected carbon became agglutinated at once and large particles were found sticking to the cells when they were withdrawn immediately after the injection. At the end of 24

hours there was still some carbon in suspension in the lymph, although this was now composed of larger particles than were present in the suspension injected. All of the mononuclear cells contained ingested carbon.

Since some of the suspension of carbon, as well as the lymph cells, is poured into the blood-stream, the blood taken from the heart of the same frog contained many more cells laden with carbon than did the lymph itself. The cells carrying carbon entered the lungs and the further phenomena occurring there were similar to those observed when the injection was made directly into the heart, except that free threads or clumps of carbon were seldom seen travelling in the blood-stream and none was deposited on the endothelium.

MacCallum (1903) described the lodging of masses of lymphoid cells and large mononuclear phagocytic cells in the lung in typhoid fever. He thought that these cells were derived from the lymph sinuses of the lymph gland and were transported through the thoracic duct into the heart and thence into the pulmonary circulation.

ACCUMULATION OF PHAGOCYTES IN THE LUNG FOLLOWING THE INJECTION OF CARBON INTO THE PERITONEAL FLUID

One-eighth of a cubic centimeter of the ink suspension was injected into the abdominal cavity of several frogs and the lung exposed one hour later. Many carbon-laden cells were already present in the lungs some of them moving with the blood-stream, others lodged along the walls of the blood vessels. Many of them were typical mononuclear leucocytes, but some were large and carried more carbon than the leucocytes. When the peritoneal fluid was examined it was found to contain some of these large cells laden with carbon which were the pre-existing clasmatocytes of the peritoneal fluid. It also contained many free particles of carbon, so it would seem that the carbon was carried into the blood-stream both free and attached to cells.

Twenty-four hours later the mononuclear leucocytes had hypertrophied and were about as large as the large cells, but they still contained less carbon.

INJECTION OF CARBON INTO THE AIR SPACES OF THE LUNG

In order to test the phagocytic power of the epithelial cells, several frogs were injected with the carbon suspension through the glottis. The ink entered the base of the lung, which was then gently massaged until the black extended to the apex. After this the lung was again inflated several times and finally distended with air and the trachea ligated to prevent collapse. When such preparations were studied under the microscope (No. 18 oc. and 16 mm. lens) the epithelial islands appeared as black spots. The black soon fell away to a great extent from the upper side of the lung and only a fine precipitate remained on the cells. This became clumped into larger granules in some regions, but not in all. After two hours there was some clotting of the carbon suspension with the mucus within the cavity of the lung, as

threads could be seen sticking to the lower septa. After a few hours some very small granules seemed to be actually within the epithelial cells. The circulation remained normal and the white blood-cells were carried along in the blood-stream without sticking to the capillary walls. No carbon appeared in the blood-stream or in the endothelial cells. There were no macrophages laden with black. In a few places an epithelial cell seemed to have been injured, as the white blood-cells lingered in this region and the injured epithelial cells seemed to be drawn back, but no carbon came through.

The following day the cells were alive and the cilia beating. The epithelial cells contained some small black granules but the islands were still intact and the cells had not rounded up as macrophages; moreover, none of them stained with neutral red in the manner characteristic of macrophages; they were still typical epithelial cells. The lung was irrigated with salt solution until all of the carbon that could be removed had been washed out. There still remained, however, many large clotted masses and threads stuck to some of the septa. The lung was then cut open in salt solution containing neutral red and examined with the No. 6 ocular and 2 mm. lens. There were only a few small carbon granules in the epithelial cells. Some of these were surrounded by a small red vacuole, so there was no doubt that the particles were within the cell. Where the cilia were active there was no carbon in the ciliated cells. The clasmatocytes, however, which lay among the ciliated cells did contain some carbon particles. In other regions of the lungs these cells contained no carbon, and there was none in the blood capillaries. The white blood-cells and endothelial cells were also free from carbon.

HANGING-DROP PREPARATIONS

In order to test *in vitro* the ability of the leucocytes to form phagocytic cells similar to those formed *in vivo*, a number of hanging-drop preparations from different frogs were studied: blood taken from the normal heart; blood withdrawn from the heart immediately following the injection of carbon; blood taken from the heart one hour and 24 hours respectively after the injection of carbon; blood taken from the heart one hour and 24 hours respectively after the injection of carbon into the dorsal lymph sac; blood taken from the heart one hour and 24 hours, respectively, after the injection of carbon into the peritoneal cavity; blood containing Kupffer cells obtained by teasing a piece of liver; blood containing spleen cells obtained by teasing a piece of spleen; normal lymph from the dorsal lymph sac; lymph from the dorsal lymph sac one hour and 24 hours, respectively, after the injection of carbon into it; normal peritoneal fluid, and peritoneal fluid taken one hour and 24 hours respectively after the injection of carbon into the abdominal cavity.

The drops of normal lymph and of peritoneal fluid contained lymphocytes, large mononuclear leucocytes, a few polymorphonuclear cells, and some clasmatocytes. After injection, the clasmatocytes took up the carbon and many of the mononuclear leuco-

cytes also ingested carbon and became transformed into phagocytic cells resembling those found in the lung.

Large phagocytic cells containing carbon were formed in the hanging-drop preparations of the blood taken from the heart, regardless of whether the carbon had been introduced into the heart, lymph sac, or abdominal cavity, the only difference being that, while little carbon was found in the cells of cultures of blood taken from the heart shortly after the introduction of carbon into that organ, considerable amounts were present in those of blood taken 24 hours after the injection of carbon into the lymph sac or abdominal cavity. The same type of large phagocytic cell was formed in all the cultures of the blood, whether carbon had been introduced or not. This cell resembled a clasmatocyte and often contained ingested red blood-cells, as described by Lewis and Lewis (1925). When carbon was present, it was never seen as a suspension in the blood but was always clumped, usually within the cells. Some of the cells contained ingested red blood-cells in addition to the carbon and there were sometimes a few clasmatocyte-like cells without carbon. When cultures of blood containing Kupffer cells were examined as soon as prepared, the Kupffer cell could readily be distinguished from the mononuclear leucocyte, because it was larger and contained the peculiar ingested material characteristic of these cells in the frog's liver. After a few days, however, the two types of cells were so much alike that the Kupffer cell could be distinguished only when it contained bilirubin crystals or some characteristic mass of ingested material.

In cultures of blood containing splenocytes there were many large phagocytic cells which could be identified as splenocytes by their characteristic ingested material, but there were also many which could not be distinguished from the macrophages originating from the mononuclear cell of the blood. There were in addition many lymphocytes and many unchanged mononuclear cells. All stages between the normal mononuclear cell and the greatly hypertrophied clasmatocyte-like cells were observed.

In all of the hanging-drop cultures there was formed a large wandering cell of the clasmatocyte type. When carbon was present, most of these cells contained carbon masses and resembled the carbon-laden phagocytes which accumulate in the lungs after the injection of carbon into the heart, lymph sac, or peritoneal cavity. It was readily seen that the carbon was not taken in as a fine suspension but as masses of different sizes, and that there was no system of pre-existing vacuoles (segregation apparatus) present in the cells in which the carbon was accumulated; but that later vacuoles sometimes were formed in these cells around the ingested particles of carbon or apart from the carbon.

When a minute amount of carbon was added to a hanging-drop of freshly drawn blood from the heart it at once began to agglutinate and formed threads and clumps of varying sizes. Whether all of the suspension became clumped when the hanging drop was shaken about depended upon the amount added, not upon the concentration of the suspension or upon

whether it was prepared in salt solution or in distilled water.

Within five minutes phenomena similar to those which occurred in the lung upon the introduction of carbon into the blood-stream took place in the hanging-drop preparations, i.e., particles of carbon were taken in by the cells (Fig. 6); the cells began to migrate on the coverglass, threads of carbon became stuck to the cells (Fig. 5), or to the coverglass, and several leucocytes became entangled with threads of carbon, forming what looked like emboli (Fig. 4). Later (24 to 48 hours) these cells hypertrophied, spread out on the coverglass, and lost their leucocytic appearance. Many of the mononuclear leucocytes divided by mitosis and even after three days some small typical mononuclear leucocytes were seen (Fig. 9), although most of them had by this time become changed.

DISCUSSION

There are vast numbers of publications upon the question of the formation of the phagocytic cells. Aschoff (1924) has just published a review of over 700 papers bearing upon this subject, and Foot has in press a review of the literature on the phagocytic cells of the lungs. Since, however, practically all of the observations of other investigators were made on mammals, in which it is impossible actually to follow the fate of the introduced foreign body, this literature will not be discussed here.

In general the results have led to two conclusions, each of which is supported by a number of investigators. First, that the phagocytic cells of the lung arise *in situ* from the endothelial cells of the lung, as claimed by Foot, Permar, and others. This, as can be shown by direct observation, does not occur in the frog, either in the lung or (as has been shown by Ponfick, 1869, and by MacCallum, 1903) in the mesenteric vessels.

MacCallum states (page 9):

> If one observes the mesenteric vessels of a frog while injecting cinnabar into the lateral abdominal vein in small quantity the red granules are seen to float free in the blood current only for a very few minutes, generally less than five, then gradually all the granules are found to be floating along surrounded by a mass of translucent protoplasm which represents the body of one or of several leucocytes which have acted as phagocytes. Such leucocyte masses with central pigment block may float in the current for a long time, finally often sticking in some small vessel. Single leucocytes with a pigment granule may emigrate from the vessel and settle themselves somewhere in the tissue.

Second, that the phagocytic cells arise immediately from the liver and spleen (Simpson, 1922). This view also does not hold for the frog, for in this form the Kupffer cells of the liver and the phagocytic cells of the spleen, bone marrow, and lymph nodes already contain ingested material by which they could readily be identified should they make their way to the lungs. Also, as pointed out by Foot (1923) and Wislocki (1924), removal of the spleen does not cause any decrease in the number of phagocytic cells found in the lungs, and as can be shown in the frog, tying off both the liver and spleen does not bring about a lessened number of carbon-laden cells in the lungs.

Some investigators describe the

origin of the phagocytic cells from the endothelium or from the liver and spleen by cell division. This phenomenon cannot account for the formation of the phagocytic cells which accumulate in the lungs within the first hour, for Levi (1916), Lewis and Lewis (1917) and Strangeways (1922) have shown that mitotic division requires over an hour; I have followed the division of mononuclear leucocytes of the frog in cultures of blood, in the lung, and in cultures of the spleen, and in each instance nearly an hour was required to complete the division, even after it was well initiated (late prophase). Whether complete division of a single cell into two daughter-cells actually occurs by amitosis is still doubtful. Nuclei have been observed to divide into two but without an accompanying division of the cytoplasm. Binucleate and even multinucleate cells have been described as separating into individual cells but without previous observation of the method of division of the nucleus. The only instance I know of in which a cell was followed during division by amitosis is that of a leucocyte of a frog described by Arnold (1887) and this required fifty minutes for completion after it was hour-glass shape. A much longer period of time, therefore, is required for either mitotic or amitotic division than is necessary for the accumulation of phagocytes in the lung.

The study of the formation of the phagocytic cells in the lung of the frog is not complicated by the presence of pre-existing clasmatocytes, as it is in the lungs of birds and mammals, for there are only a few of these cells and they are easily identified by reason of their ingested material (red blood-cells and melanin granules). In the frog there are no dust cells, but when carbon is introduced into the air-spaces some of the clasmatocytes lying between the ciliated cells may take up some of the carbon, in which case they come to resemble the dust cells of higher forms. The mononuclear leucocyte, however, after lodging in the lungs, hypertrophies, the nucleus becomes changed, and the final form resembles (except in the type of ingested material) the clasmatocyte; so that, as Wislocki states, nearly all the carbon in the lungs is taken up in the cytoplasm of cells which possess all the characteristics of clasmatocytes. That these cells arise from leucocytes can readily be demonstrated by following their entrance and subsequent behavior in the living lung of the frog, or, as shown by Lewis and Lewis, by following hanging-drop cultures of blood itself.

It is most surprising that previous observers have taken it for granted that the suspension of carbon remains a suspension after it is injected into the blood-stream, especially since certain investigators lay great stress upon the necessity of preparing the suspension in distilled water. Upon this surmise have been built unwarranted conclusions in regard to phagocytosis in general, such as the ingestion of ultramicroscopic particles and the existence of a segregation apparatus. The observations of colloidal chemists should warn one to expect some sort of a change when a suspension is brought into contact with a colloidal solution of a different salt content and a different hydrogen-ion concentration. There is reason

to expect some agglutination of the carbon particles to take place, as can readily be demonstrated when a drop of blood and a drop of suspension of ink are brought together. Buxton and Torrey (1906) described the formation of a coagulum over the surface of the peritoneal cavity following the injection of particles such as bacteria and lamp-black. Addison and Thorington (1918) mention the possible agglutination of the colloidal aggregates of trypan blue due to the various electrolytes present in the peritoneal fluid and the later ingestion of the larger particles by the cells. That there is no preformed apparatus for the segregation of ingested material existing in cells of tissue cultures or those of the blood has already been demonstrated (Lewis and Lewis, 1915; Lewis, 1919; Prigosen, 1921; Smith, 1921; Lewis, 1923) and is once again shown in the present experiments; for if, shortly after injection, the lung is stained by adding neutral red to the salt solution used for moistening it, the leucocytes seldom contain any red vacuoles either free from carbon or around the ingested particles.

There is an intimate relationship between the lymph and the blood, and also between the blood and the peritoneal fluid, so that when foreign bodies are introduced into the lymph sac or the abdominal cavity they very soon enter the blood-stream. Buxton and Torrey also found that in the rabbit, when a foreign substance was injected into the peritoneal cavity, it passed to the lymph channels of the diaphragm, to the lymph nodes, and by way of the thoracic duct to the circulating blood, so that the particles reached the blood current shortly after injection.

That a leucopenia is brought about by the introduction of foreign bodies into the blood-stream has been shown by a number of observers. Tchistovich (1899) found that after intravenous injection of streptococci into a rabbit there was a decrease in the number of leucocytes of the blood, because the leucocytes ingested the cocci and became arrested in the capillaries of the lungs. Bull (1915) states that when typhoid bacilli were injected into the ear vein of a normal rabbit they were agglutinated within thirty seconds, and that within seven minutes they were removed from the blood-stream and lodged in the sinusoids and blood-spaces of the liver, spleen and lungs, where already a large proportion of the clumps had been ingested by leucocytes which congregated in these organs following the injection.

The solution of the problem concerning the origin of the phagocytic cells of the lung has been greatly complicated by the type of experiment used by the various investigators. Some have examined the animal one hour after a single injection of carbon, others not for several days. Some have given several injections of carbon, others have used numerous injections of vital dyes preceded or followed by an injection of carbon or other particulate matter. Foot and Permar have combined carbon injection with pathogenic bacteria. The results obtained must depend largely upon what cells are brought in contact with the foreign body and how much secondary breaking down of cells takes place. Moreover, the simple fact that a cell has ingested some of the foreign body does not mean that it necessarily becomes transformed into the type of cell that

most investigators imply in the term *macrophage* or *phagocytic* cell; for, as shown by Smith (1921), and Smith, Willis, and Lewis (1922), almost all types of cells may ingest foreign bodies without necessarily altering their character. In these observations upon the living frog no attempt was made to enter into any more complicated a question than the simple origin of the wandering phagocytic cells which appear in the lung immediately following an introduction of carbon into the blood-stream. A comparison of figures and the description of the phagocytic cells formed in the lungs of rabbits following the introduction of carbon into the circulation shows that these cells correspond to those formed in the lungs of the frog, not only in appearance but also in some instances in time of origin. Since, in the frog, it is possible actually to follow their origin *in vivo*, it would seem as though these observations might throw light upon the formation of the macrophages found in lungs in general.

CONCLUSION

The phagocytic cells which accumulate in the lung of the frog following the introduction of carbon originate from leucocytes of the circulating blood and not from the endothelium *in situ*, nor as showers of macrophages from the spleen and liver.

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On the Alleged Power of Bacteria to Form Bile Pigment from Haemoglobin

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In the literature concerned with the formation of bile pigment one meets with the statement that haemoglobin can be changed into bilirubin by the action of bacteria. Quite recently this belief has appeared in the form of a criticism of experiments in which laked blood injected into animals, in order to study the site of origin of bile pigment, was subsequently allowed to stand for some hours exposed to the air before being tested for bilirubin (1). However, one can find very few recorded observations on the transformation of haemoglobin into bilirubin by bacterial action. In 1908, Hollis (2) reported that he was able to detect bilirubin in laked blood left standing for some days exposed to the activity of air bacteria, but he fails to mention whether tests for bilirubin had been carried out upon this blood before exposure to the air. In 1910, Herzfeld and Steiger (3) reported that the haemoglobin of laked blood exposed to the action of the Fränkel pneumococcus becomes converted into bilirubin within several days. Leupold (4) and Hueck (5), on the other hand, state that they found no bilirubin crystals in the sediment which forms in blood exposed to bacterial action. Neither of these investigators tested the supernatant fluid for bile pigment, however, and Hueck remarks that one has still to reckon with the possibility that in their experiments it may have actually been present in solution. Thus, the statement that haemoglobin can be changed to bilirubin by bacteria rests upon the incompletely described experiment of Hollis and the more definite report of Herzfeld and Steiger.

Although we have ample evidence that haemoglobin can be changed into bile pigment within the living body, we know practically nothing of the mechanism, or of the steps, by which this transformation is brought about. Especially are we uncertain whether or not the process can take place within the body fluids outside of living cells. At the beginning of a search for a bile-pigment forming enzyme, the existence of which is so commonly postulated in books and articles dealing with the formation of bile pigment (6), it was thought advisable to attempt to study first the manner in which bacteria produce this pigment from haemoglobin. However, as the following experiments will show, we have not been able to confirm the statement that bacteria have any such power. In no instance has bile pigment appeared in detectable

amount after the inoculation of haemoglobin or laked blood with bacteria of different types.

EXPERIMENTS

Whole and laked blood. Blood was withdrawn under aseptic precautions from the jugular vein of a dog into a sterile flask containing glass beads, and defibrinated. Of this defibrinated blood 8 cc. were introduced into each of four sterile test-tubes. To the first tube 0.5 cc. of sterile bouillon was added; to the second, 0.5 cc. of a 24-hour bouillon culture of Type I pneumococcus; to the third, 0.5 cc. of a staphylococcus aureus culture; and to the fourth, 0.5 cc. of a culture of streptococcus viridans. To the remainder of the defibrinated blood an equal amount of sterile distilled water was added in order to lake the red cells. Of this laked blood 10 cc. was put into each of four sterile tubes and these tubes were inoculated in the same manner as the previous set. After four days' incubation at 37° a control culture was taken from each tube and the contents were subjected to the following tests for bilirubin:

1. van den Bergh's indirect diazo-reaction: In the presence of excessive haemolysis it is often difficult or impossible to detect minute amounts of bilirubin by this test, for after precipitation of the proteins the alcoholic solution may be colored by haemoglobin. Dilution will serve to lessen greatly this disturbing factor, but at the same time may dilute minute amounts of bilirubin to a point below the limits of detection by the test. However, since the test is sensitive to 1:1,500,000 parts of bilirubin, if bilirubin is present in even moderate amounts, quite considerable dilution may be resorted to before such a point is reached. As a control, an equivalent amount of a mixture of HCl and sodium nitrite, prepared in the proportions in which these substances are used in the van den Bergh test, was always applied to a portion of the solution for comparison with the van den Bergh test. This control, which represents the van den Bergh reagent *minus* sulphanilic acid, and acts as an acid oxidizing reagent, turns a solution of bilirubin green instead of pink, and serves to demonstrate whether any change in color occurring in the solution to which the van den Bergh test is applied represents a diazo-reaction, an oxidation, or an acid effect. Both the control solution and the diazo-reagent decolorize a given haemoglobin-stained fluid in exactly the same degree in the absence of bilirubin. A further control in which a small amount of bilirubin was added to the solution to be tested, served to demonstrate the concentration at which bilirubin could be detected in the solution under consideration. In addition, the diazo-reagent was always controlled with a solution of bilirubin alone.

2. The Gmelin test, always carried out by layering the test fluid and reagent.

3. Extraction with warm chloroform. The sediment and fluid remaining after the van den Bergh and the Gmelin test had been carried out was shaken with warm chloroform and the chloroform portion then separated and examined for a yellow color. Bilirubin dissolves readily in chloroform with an intensely orange yellow

color which is quite distinct in dilution even as great as 1:500,000.

Table I shows the results of these tests.

In all cases, repeated van den Bergh tests were entirely negative and the chloroform extractions of the various solutions were quite colorless. However, when these solutions were layered with nitrous acid in the Gmelin test, a distinct greenish ring slowly made its appearance in each case; but the play of colors characteristic of bilirubin, although watched for closely, was never seen. We have made no attempt to determine upon what substance the appearance of the green ring depends, but in view of the negative van den Bergh test, the chloroform extraction test, and the failure of the other characteristic colors of the Gmelin test to appear, it is clear that the substance causing the green ring was not bilirubin. Indeed, it was found that, when laked blood was allowed to stand for several hours under a variety of conditions, the green ring would appear on treatment

TABLE I

	VAN DEN BERGH	GMELIN	CHLOROFORM EXTRACTION	CONTROL CULTURE
Sterile blood	0	Green ring	Colorless	No growth
Blood + staph. aureus	0	Green ring	Colorless	Pure culture of staph. aureus
Blood + strept. viridans	0	Green ring	Colorless	Pure culture of strept. viridans
Blood + pneumococcus (Type I)	0	Green ring	Colorless	No growth
Sterile laked blood	0	Green ring	Colorless	No growth
Laked blood + staph. aureus	0	Green ring	Colorless	Pure culture of staph. aureus
Laked blood + strept. viridans	0	Green ring	Colorless	Pure culture of strept. viridans
Laked blood + pneumococcus (Type I)	0	Green ring	Colorless	No growth

TABLE II

TIME	VAN DEN BERGH	GMELIN	HUPPERT	CONTROL CULTURE
Whole blood with pneumococcus (Type 1)				
days				
2	0	Green ring	0	Pneumococci in pure culture
4	0	Green ring	0	Pneumococci in pure culture
9	0	Green ring	0	Pneumococci in pure culture
Laked blood with pneumococcus (Type 1)				
2	0	Green ring	0	Pure culture pneumococcus
4	0	Green ring	0	Pure culture pneumococcus
9	0	Green ring	0	Pure culture pneumococcus

with nitrous acid. Unless the play of colors of the Gmelin test were carefully watched for, this ring was often striking enough to be mistaken for a positive test for bilirubin. It seems possible that this source of error may have played a rôle in the experiments of Hollis and of Herzfeld and Steiger.

Since no living pneumococci were found in the haemoglobin after four days, the experiment was repeated, 5 cc. of the bouillon culture instead of 0.5 cc. being employed. The contents of the tubes were examined after two, four and nine days' incubation, and living pneumococci were found abundantly. In this experiment the Huppert test was substituted for the chloroform extraction, but it was found to be rather unsatisfactory in the presence of large amounts of free haemoglobin.

Armour's haemoglobin. A one-half of one per cent solution of Armour's haemoglobin was filtered through a Berkefeld filter and kept for 24 hours in the thermostat, after which it was cultured and found to be sterile. Of this solution 8 cc. were placed in each of five tubes. To the first was added 4 cc. of a bouillon culture of staphylococcus aureus; to the second the same amount of streptococcus viridans; to the third, Type I pneumococcus; and to the fourth, 4 cc. sterile bouillon; to the fifth the same amount of sterile bouillon, after which the contents of this tube were left exposed to the air. These solutions were examined after three days' incubation at 37°C. In this and in the succeeding experiment no sediment formed and the van den Bergh test was so clearly negative that extraction with

TABLE III

	VAN DEN BERGH	GMELIN	CONTROL CULTURE
Haemoglobin and staph. aureus	0	Green ring	Staph. aureus in pure culture
Haemoglobin and streptococcus viridans	0	Green ring	Strept. viridans in pure culture
Haemoglobin and pneumococcus (Type I)	0	Green ring	Pneumococcus (Type I) in pure culture
Haemoglobin and bouillon (sterile)	0	Green ring	No growth
Haemoglobin and bouillon (exposed to air)	0	Green ring	Mixed flora

TABLE IV

	TIME	VAN DEN BERGH	GMELIN	CONTROL CULTURE
Haemoglobin and strept. viridans	24 hours	0	Green ring	Strept. viridans pure culture
Haemoglobin and strept. viridans	4 days	0	Green ring	Not cultured
Haemoglobin and pneumococcus (Type I)	24 hours	0	Green ring	Pneumococcus (Type I) pure culture
Haemoglobin and pneumococcus (Type I)	4 days	0	Green ring	Not cultured

chloroform was not carried out (Table III).

Merck's haemoglobin. A one-half of one per cent solution of Merck's haemoglobin was passed through a Berkefeld filter, incubated at 37° for 18 hours and a culture taken, which showed no growth in 24 hours. Into each of four tubes were put 5 cc. of this sterile haemoglobin solution. Of a bouillon culture of pneumococcus Type I 5 cc. were added to two of the tubes and 5 cc. of a culture of streptococcus viridans were added to the other two tubes. These tubes were incubated at 37° and tested as before.

Dog's blood + oxygen. On the assumption that the conversion of haemoglobin into bilirubin might depend upon the action of an oxidase, we have supplied an excess of oxygen to sterile citrated blood incubated with an equal amount of bouillon cultures of pneumococcus (Type I). Oxygen was bubbled through this solution at intervals, but at the end of eighteen hours no bilirubin could be detected by either the van den Bergh test or by extraction with chloroform. Cultures taken at the end of this time showed viable pneumococci.

SUMMARY

Whole blood, laked blood and solutions of haemoglobin subjected to the action of the pneumococcus (Type I), staphylococcus aureus, streptococcus viridans and air bacteria were tested for bilirubin after various intervals of incubation. No bilirubin was formed in amounts detectable by the van den Bergh test, the Gmelin test, or by extraction with warm chloroform.

After these bacteria have been allowed to act upon haemoglobin, if the solution is layered upon nitrous acid (Gmelin test) the green ring which appears does not indicate the presence of bilirubin in the solution. The play of colors characteristic of the Gmelin test for bilirubin does not appear, and other more sensitive tests are negative for bilirubin. It is possible that this atypical reaction may have played a rôle in the conclusions of previous investigators, who have stated that bilirubin can be readily formed from haemoglobin by bacterial action.

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The Johns Hopkins Medical Research Club

Abstracts of Papers Presented

Note on the Combination of Dyes and Proteins. By ARTHUR GROLLMAN. *Presented March 20, 1925.*

Despite the many applications of dyes to biological problems no quantitative study has been made of their combination with proteins and other biologically important systems. At the suggestion of Professor E. K. Marshall, Jr., a study of the combination of phenolsulphonphthalein (phenol red) and proteins was undertaken.

In the case of charcoal (used as an inert comparison substance), casein (technical and C. P.), and undissolved gelatin, this combination was studied by the method usually employed in determining adsorption from solution by solids. Hydrophilic systems, such as solutions of blood albumin, egg albumin, blood serum, tissue extracts, etc., were studied by means of ultrafiltration. In this process separation of the intermicellar fluid from the dissolved colloids permits the determination of the amount of dye held by the latter. The results obtained with all of these substances could be expressed by the usual adsorption formula:

$$\frac{x}{m} = KC^{1/n}$$

where x is the amount of dye combined with m grams of absorbent, C, the equilibrium concentration, and K and $1/n$ are constants. The characteristic feature of adsorption on proteins is the high value of $1/n$ as compared with that of inert substances like charcoal.

Variation in the pH of the dye solution has an effect on the adsorption on proteins entirely different from that on charcoal. In the latter substance, increasing the pH causes a continuous decrease in the adsorption. With proteins, on the other hand, there is a maximum on the acid side of the isolectric point. This behaviour in the case of the proteins is attributable to their change in dispersivity and chemical properties by the addition of acid or alkali. An increased hydration with the formation of the protein salts may also account—in part at least—for this decrease in adsorption. Indeed, in extreme alkaline solutions, the concentration of the ultrafiltrate from an original albumin solution containing only 60 mgm. of phenol red per liter may be 90 mgm. per liter. This highly negative adsorption would indicate the binding of considerable water by the albumin. An investigation of this problem of the hydration of proteins is now being further studied by other methods.

A study of the adsorption of phenol red by the sera of various animal species—rabbit, dog, pig, hen, duck and frog—shows the variation in binding power of the sera of these sources to be due to the variation in albumin content, as would be expected from the results with the individual protein constituents. For the same reason, the amount of free dye present in the blood of patients with a low albumin content will be much greater than in the normal, and since it is this free dye which is the active concentration with which the tissues must deal, it is evident that this factor must be considered as one of the extra-renal influences which renders the phenolsulphonphthalein test unreliable in many clinical cases.

Book Reviews

Angina Pectoris. By Sir James Mackenzie, M.D., F.R.S., F.R.C.P., LL.D. Ab. and Ed., F.R.C.P.I. (Hon.) 1923. 253 pages. $9.00. (*Henry Frowde; Hodder & Stoughton, London.*) (*Oxford University Press, New York.*)

This last volume of Sir James Mackenzie's is an exposition of his well known views upon the nature and pathology of angina pectoris, discussing in great detail the significance of all the symptoms associated with the clinical evolution of this morbid state. To those readers who know the author's previous writings there will be found little that is new in this text, which represents his conception of the underlying pathology upon the basis of what may be called its myocardial origin, as distinct from that group of cases regarded by some as associated with primary aortic disease. He also emphasizes his opinion as to the two types of angina pectoris: the one primary, due to organic changes in the heart, incurable, but which may progress slowly or rapidly; the other which he calls secondary, an expression of disturbances due to reflex processes in hypersensitive states and wholly curable.

One interesting point stressed again, as so often in the past, is the curious antagonism, if we may call it such, existing between the incidence of angina pectoris and auricular fibrillation and complete heart block. Angina pectoris has never been encountered by the author in association with complete A-V dissociation or in something over 500 cases of auricular fibrillation.

We wish this volume might have been offered to the profession in approximately half the number of pages and at half the cost. The chapter entitled Neurasthenia and the case records might have been omitted without sacrificing anything of value, and in many places the text might be condensed to great advantage. As a more compact volume we are confident that its teachings, fundamentally sound and of great value, would have reached a much larger number of readers. Anyone turning to this text in the hope of finding support for the very modern surgical procedures in the treatment of angina will be disappointed.

Since the above note was written we have learned of the death of Sir James Mackenzie. As has so often happened, the master is the victim of the very disease to which he has devoted a life's study. We are under an immense debt to Sir James Mackenzie for his leadership, his enthusiasm and simplicity and above all for his soundness and clearness of exposition in his chosen field. His death closes an extraordinarily interesting and distinguished career, which, measured by the good accomplished during a long and active life, cannot be over estimated.

E. P. C.

Practical Anesthetics. By H. Edmund G. Boyle and C. Langton Hewer. $2.00. (*Oxford Medical Publications. Henry Frowde; Hodder & Stoughton, London.*)

The first edition of this book appeared in 1907. We read in the preface that "The book has been almost completely re-written and brought up to date." In fifteen chapters and 172 pages the authors have touched on every aspect of anesthesia, and, though brief, the book gives a very concise, clear picture of the subject. There is nothing distinctly new in the text. As usual in books on anesthesia, written by British authors, the use of chloroform, ethyl chloride and ethanesal is emphasized. Ether is not so popular in England as in America. At the same time, the dangers of chloroform and ethyl chloride are not disregarded. We read in Chapter X that chloroform can cause death in at least

three ways: (*a*) by primary cardiac failure, (*b*) by respiratory failure, (*c*) by so-called "delayed poisoning."

Dr. E. I. McKesson contributes Chapter IV to the book—four pages on Blood Pressure and Pulse Reactions during anesthesia.

M. B.

Principles of General Physiology. By Sir William Maddock Bayliss. 4th edition. $8.50. (*Longmans, Green & Co., London, 1924.*)

This edition "has been revised and prepared by a Committee" of twenty-four friends whose names appear in the preface (Prof. A. V. Hill, editor), "as a small but sincere tribute of regard and sympathy for Sir William Bayliss at the time of his present sickness," a sickness that terminated in the author's death before he could see the completed final proof.

Piety and affection obviously stayed the hand, as often as zeal prompted it, in the work of editing this posthumous volume. The book thus is unchanged, so far as being a reflection of the character and opinions of the author. Faults and errors as well as virtues have been preserved, if not magnified, even to the point where they concern publications of the individuals of the editorial committee. This treatment, together with the very delightful portrait of the author surreptitiously inserted by the committee as frontispiece, leaves this, the most human of all books on the principles of a science, a speaking memorial to the late professor of general physiology in University College, London.

C. D. S.

Local Anesthesia Simplified. By John Jacob Posner, D.D.S. $3.50. (*C. B. Mosby Co., St. Louis, Mo.*)

In the preparation of this interesting and instructive book, the author does not delve into odd or ancient methods of anesthetizing the teeth. He states that the purpose of the book is to eliminate fear of the dental chair, both in extraction and in the preparation of cavities in sensitive teeth, as well as to simplify the technique to a thorough working basis.

There is a short but interesting chapter on the discovery of cocaine and its introduction into dental practice some forty years ago—Dr. William Stewart Halsted, of Johns Hopkins University, being the first to use the nerve-blocking method of anesthetizing the mandible. More recently, novocaine has almost entirely replaced the highly toxic cocaine.

The procedure described in Dr. Posner's book has been so simplified, that there are only two methods necessary to anesthetize all teeth in either the maxilla or mandible. Owing to the difference in the bone structure of the upper and lower jaws, the author tells why his supraperiosteal method is the best for the upper jaw, and the nerve-blocking mandibular injection the best for the lower jaw. There are many fine illustrations devoted to these two methods of anesthetizing the teeth.

For those who wish to obtain a thorough understanding of local anesthesia as applied to dentistry, this book covers the field in a very thorough manner.

G. A. B.

Physiological Principles in Treatment. By W. Langdon Brown. Fifth edition. $3.75. (*New York, William Wood and Co., 1925.*)

In the presend edition two new chapters have been included—one on the work of the liver, the other on asthma; while new sections have been added on fractional test-meals, insulin, uraemia, and dyspnoea.

To American readers the book affords a good opportunity of contrasting the methods of dealing with disease in use in England and in their own country. The principles come out clearly as the same, but many of the details employed in making use of them are different. Some methods firmly established in American medicine are lightly touched on, while others described, and in common use in Britain, are scarcely practised in America at all. No American text-book of this kind could be written without consideration of the newer work on nutrition and basal metabolism in its application to the investigation and treatment of disease, and yet in this book these developments are omitted.

Diabetes and the new problems introduced with the advent of insulin are thoroughly dealt with, and the dietetic treatment advocated by Dr. George Graham is given prominence and approval.

For American readers the chapters on uric acid, the purin bodies, albuminuria and the treatment of nephritis, and intestinal intoxications will all give an outlook on such problems from a different angle, which cannot help being stimulating.

It is this traditional and national difference in outlook, when interpreting the same physiological principles in the treatment of disease, that should make this small book very welcome in the United States.

J. W. M.

Pediatrics. Isaac A. Abt, Editor. Vol. IV. $10.00. (*W. B. Saunders Co., Philadelphia and London.*)

The fourth volume of Abt's Pediatrics maintains the high standard set by the earlier volumes of the work, which will unquestionably long remain the standard large reference textbook of its kind. All its virtues as well as its faults arise from the method of its compilation, namely, the aggregation of complete articles on definite subjects by leading authorities, regardless of their appropriateness in a work on pediatrics. To illustrate, of the twenty-six contributors, twelve (and among these several of the best known) are not pediatrists at all. On the other hand,if the book be regarded as a source of the latest knowledge needed by student or practitioner of pediatrics, it is undeniably useful. For example, the articles on *Electrocardiography in Children* and *The Urine in Infancy and Childhood* are small monographs on the subjects. The discussion of the endocrinopathies are as fascinating as they are valuable. The five chief heads: circulation, blood, endocrine glands, kidney, and genitalia, are admirably handled as to thoroughness and proportion; the many charts, tables, and diagrams contribute to the clearness and practical value of the articles; and the bibliographies are frequent, full, and as nearly modern as can be expected. Numerous condensed case histories add to the interest.

G. W. R.

Pediatrics. Isaac A. Abt, Editor. Vol. V. $10.00. (*W. B. Saunders Co., Philadelphia and London., 1924*).

Volume V is largely devoted to orthopedic subjects but in addition contains chapters on tuberculosis, hereditary syphilis, and a number of the tropical diseases. The article on tuberculosis is of special excellence, It is to the section on orthopedics that the pediatrist will wish to refer for information and guidance on many points which come up in his practice. These chapters are written by a number of well recognized authorities. One must regret, however, that the subject is approached from the point of view of the orthopedist, rather than that of the pediatrist. A number of diseases which the pediatrist must constantly be prepared to recognize are not discussed. There is no mention of acute epiphysitis, while Perthe's disease and Still's disease are not described. There is no discussion of the bone lesions in scurvy. On the other hand, there is much material which does not concern the pediatrist—chapters on traumatic dislocation, the surgery of tendons and the reconstructive surgery of infantile paralysis. The present volume, which contains so much excellent material, could be made a still more valuable addition to the pediatrist's shelf by a more careful selection of the material, especially that which would help him to solve the particular problems with which he is confronted.

L. W.

Principles of Psychotherapy. By Dr. Pierre Janet. Translated by H. M. & E. R. Guthrie. $3.00. (*The MacMillan Company, New York, 1924.*)

In a very readable short volume Janet presents both an historical and personal approach to psychotherapy. The historical section which clearly and entertainingly traces the development of the psychological cures by miracles, "animal magnetism," hypnotism, reëducation, faith and works, Christian Science, moral suasion, "rest"

cures, etc., is fairly and scientifically presented. A second part devoted to the Principles of Psychotherapy is an exposition of his own psychology of the neuroses. His well known views on suggestion, somnambulism, hypnosis, and psychic tension are well summarized and his notion of a reinvestment of psychic energy in patients apparently bankrupt by "stimulation" is rhetorically persuasive. A third part devoted to "The results of psychotherapy" has the virtue of being scientifically sceptical but hopeful.

His psychology is a curious admixture of "faculty" psychology, a professed behaviorism, and hypnosis jargon. To an American public it will probably seem archaic. His treatment of Freudian methods is inadequate and, to the reviewer, unfair. He classifies Psycho-analysis as a cure derived from animal magnetism, and although insisting that Freud took over bodily his own ideas, he denies practically all of their validity.

The book represents the best of the French method of approach and is to be recommended to those who have a historical interest in medicine as well as in psychotherapy.

L. B. H.

Chronologia Medica. By Sir D'Arcy Power, K.B.E., and C. J. S. Thompson, M.B.E. 278 pages. $3.50. (*Paul B. Hoeber, Inc., New York City.*)

A chronology at best cannot aspire to more than a very brief sketch of the material with which it deals. This book has been written with the expressed endeavor of serving as a "handlist of the chief persons, periods, and events in the history of the healing art from traditional times . . . . to the present time." The authors have attempted to assign dates wherever possible, these being the basis of arrangement of the material. An index at the end makes possible a cross reference which adds considerably to the utility of the work.

The chief merits of the book lie in its comprehensive scope and its concise style. Each character is introduced with several telling words which place him immediately as anatomist, philosopher, physician or surgeon, and there are illustrations of many of the more prominent figures. The accounts in the main are readable and generally contain appropriate facts of more than passing interest which help to place the book outside of the class of a mere reference volume. One wishes for more details in many instances but can scarcely hope for them in such limited space.

At the end of the book there are nine pages given over to a chronology of drugs, a very brief but enlightening account of their discovery or their earliest mention. These are arranged, unfortunately, according to dates, without any index, which makes the material quite unavailable for quick use. One is rather surprised to see no mention of digitalis, though it is noted in the brief account of Withering. Finally, there is a chronology of the principal universities and schools of medicine with approximate dates of their founding, incidentally omitting the first American university established for graduate study. At the end there is a bibliography of the chief works on the history of medicine.

The book should appeal strongly as an introductory guide and reference volume to anyone interested in the history of medical sciences. It does not purpose to give details, but it is comprehensive and contains in compact form an abundance of very useful material.

M. N. F.

Gynecology with Obstetrics. A Textbook for Students and Practitioners. By John S. Fairbairn, M.A., B.M., B.Ch. (Oxon), F.R.C.P. (Lond.), F.R.C.S. (Eng.). 769 pp. 8°. $8.00. (*Oxford University Press, London, 1925.*)

It is the general plan rather than the actual substance of this book which invites comment. It consists of eight parts. Part I (Anatomy and Physiology), Part II (Pregnancy), Part III (Labor), Part IV (Lying-in Period), Part V (New-born Infant), the first half of Part VII (Obstetrical Operations) and Part VIII (Public Health Aspects) are solidly obstetrical, although Part I is of course of equal gynecological interest. On the other hand, Part I, together with Part VI (Diseases of the Reproductive Tract) and the second part of Part

VII (Gynecological Operations) are entirely gynecological. The combination of the two subjects thus constitutes a somewhat lumpy mixture rather than a smooth blend. The author has merely crowded, between the covers of one volume, a textbook on obstetrics and one on gynecology. Regardless of what one's opinion may be of the general wisdom of combining the two subjects in teaching and practice, this work is not a convincing argument for combining them for textbook purposes.

The general subject matter is admirably treated, the author's style being clear and pleasing. The discussion of such fundamental topics as menstruation and ovulation, topics of real borderline importance, is perfunctory and inadequate. The sparsity of illustrations throughout the book is regrettable. The effort at crowding two big subjects into one moderate-sized book has inevitably laid the author open to the charge of disproportion in the treatment of subjects. Gynecological operative procedures are disposed of in less than nine pages, obstetrical operations in twenty-five, and all without a single illustration. And yet the author devotes Part V (45 pages) entirely to pediatrics, including a discussion of artificial feeding. Certain chapters might be singled out as especially commendable, such as those on abortion and ectopic pregnancy. Not a single reference is given, and a number of important recent procedures, such as tubal insufflation for testing tube patency, are not mentioned. And yet all the subjects are treated in such a pleasing, business-like fashion, and the author's attitude on all matters is so unimpeachably sensible and so obviously based on a large experience, that the everyday reader will read it with profit and pleasure, and quite possibly may not be impressed with the few objections indicated by the reviewer.

E. N.

Inanition and Malnutrition: Its Effects upon Growth and Structure. By C. M. Jackson. 616 pp. $8.00. (*P. Blakiston's Son & Co., Philadelphia.*)

This is an exhaustive treatise on morphological changes dependent upon varying degrees of food deficiency. The study is the result of years of interest, reading, and personal work by one who stands preeminent in his field. One of the chief criticisms is that the treatment of the subject is purely morphological. The author assiduously avoids function as though it were unclean food. The trend in modern science to unite morphology with function appears to us to be a wise one. To divorce them completely, as Professor Jackson has done, seems unnatural and strained. The work is a review of more than 2500 articles. One feels that the evident decision of the author not to comment upon the relative value of the references is a mistake; good and bad, ancient and modern are given equal place. One feels, too, that the author, with his interest and wide experience, could afford to declare his preferences on controversial points more freely. The chapter on the effect of inanition and malnutrition on the female reproductive system seems to us to be one of the best, perhaps because here the author discusses function as well as form.

The composition is laboriously arranged. The book makes rather tedious reading. Everywhere fluency of style and dramatic interest are sacrificed to pure statistical precision. The chapters all follow a set skeleton—the repetition of form, to say nothing of a too frequent repetition of content, is monotonous. The frequent quotation of passages in French, German, and Italian seems unnecessary.

One must admire the completeness of the work and the tremendous amount of labor which forms its foundation. It deals not only with man but with the entire biological realm.

The book is assured a valuable place as a source of reference. With its bibliography or more than 100 pages, the excellent index, and the tables of organ weights, one is able to find complete data with facility. But the work is mainly a review of the literature and its greatest appeal must be for persons working directly with the subject.

Hence we feel that we have here an important contribution, with a valued place in the office of the medical statistician but hardly in the physician's library.

M. S. G.

Operative Surgery. By J. SHELTON HORSLEY, M.D., F.A.C.S. Attending Surgeon, St. Elizabeth's Hospital, Richmond, Va. 784 pp. $12.50. (*C. V. Mosby Co., St. Louis, 1924.*)

A pleasing thing about this book is its size. It is an easy volume to handle and is, therefore, in striking contrast to the many heavy and cumbersome volumes that are to be found in the surgeon's library. The illustrations are excellent; they are very clear and simple in line and form, and may be understood almost at a glance. They are an argument for the advantage of good drawings over photographs. The written matter is clear and concise; the sentences are short and direct and the reader is never in doubt as to the author's exact meaning. Very often the writer limits himself to the description of some one operative procedure as in pericardiotomy. Still, the book is not entirely a personal surgery, as it abounds throughout with many references to other men and their methods, as for instance, the Finney pyloroplasty and Kerr's method of resection of the intestine. Very noticeable, also, is the almost total absence of any description of "Classical Operations." The book concerns itself only with surgery as practised today and is not filled with a lot of copied descriptions of operative procedures.

It is to be hoped that any future edition will be maintained as an expression of the author's personal experience, and not later amplified into an encyclopedia of surgery, in which the author's personality may be almost entirely lost.

A. S.

Surgical Pathology. By WILLIAM BOYD, M.D., M.R.C.P. (Ed.), F.R.S.C. 837 pp. $10.00. (*W. B. Saunders Company, Philadelphia, Pa.*)

This book will have its greatest value when used by students in conjunction with more exhaustive and complete works on general pathology. It succeeds admirably in bridging a gap between purely pathological and clinical presentations of disease processes For those interested primarily in surgical pathology, the material is presented in a convenient form, but often not as much in detail as might be desired.

One quarter of the book is given over to general discussions of surgical conditions. In the remainder, these conditions are described as they affect particular organs and systems. Many of these discussions are very brief, but this brevity is compensated for by the choice of references cited after each chapter. The more common surgical lesions seen in lymph nodes, the breast, and in uterine curettings, merit, however, more complete exposition. The illustrations, the majority of which are not original, are satisfactory.

Because of the material covered, and the manner of its presentation, this book is a welcome addition to the list of text-books on pathology, and one which may be heartily recommended.

E. M. H., Jr.

Proceedings of the International Conference on Health Problems in Tropical America, under the Auspices of the Medical Department of the United Fruit Company, Kingston, Jamaica, July 23-31, 1924.

This is a book of about one thousand pages, well bound in cloth, well printed on light paper and containing a number of excellent plates, some in color. It is a worthy record of an unusual meeting. The meeting would have been a credit to any Government or any large Medical Association, but organized as it was by a private Corporation, it was a striking example of the Company's confidence in its own medical officers and in the financial value of Tropical Medicine and Sanitation.

Dr. Deeks, General Superintendent of the Medical Department, planned originally to have a meeting of his medical superintendents with addresses by a few guests, but the party grew until it included such leaders in tropical and general medicine as Agramonte, Ashford, Banting, Bass, Carter, Castellani, Fülleborn, Harris, Hoffman, Kofoid, Lane, Noguchi, Park, Rogers, Rosenau, Simmons, Stephens, and many others—to the number of seventy, whose papers make up the volume. An unusually good system of reporting discussions adds to the value of the book, by giving the last word in the opinion of competent observers.

The following subjects are particularly well covered: Malaria, Amoebic Dysentery,

Yellow Fever, the Deficiency Diseases including Pellagra, Sprue, etc., and various skin diseases. The material is arranged systematically.

The publication of this book must be considered an event in American Tropical Medicine and will undoubtedly stimulate the subject, not only in the Medical Department of the United Fruit Company but among workers in all the Americas.

In addition to the regular meetings, an important part of the trip was the visits to the different plantations and hospitals bordering on the Carribean. Naturally these are difficult to record and could only be appreciated by those who were fortunate enough to attend. As Castellani said at a farewell dinner—"Encore!"

H. J. N.

Blood Pressure: Cause, Effect and Remedy. By Llewellys F. Barker and Norman B. Cole. pp. 154. $1.25. (*D. Appleton and Company, New York, 1924.*)

It is not an easy thing to present a medical subject to the general public clearly. Nor is it easy to expound a subject, whose delimitations are not clear cut, in such a way that all sorts and conditions of readers may receive just the correction impression. The abnormalities of blood pressure especially need nice handling in presentation.

The authors give a good deal of space to the detailed account of the anatomy and physiology of the cardiovascular system. This description is very clear and any careful reader will be able to obtain a very good mental picture of the situation, both normal and abnormal.

The causes of hypertension they divide roughly into those of functional and organic origin. They are inclined to think, however, that even in functional disturbances structural changes (in which chemical changes are included) may be present.

The same clarity of description continues in the chapter on the secondary effects of hypertension, and though the reader is exhorted not to "distress" himself "with too vivid contemplation of the inevitable final result to the patient" one feels that it might be kinder to admonish him to omit Chapter VII, especially if his blood pressure is much above the average level. However, he is soon in placid waters again, because, after a short note on conditions of hypotension there is an excellent chapter on "What to do?". This is really a summary of the essentials of a sensible mode of living for everyone and, quite apart from the particular considerations of those with hypertension, could be studied by the general public with great profit.

T. O.

Report on the Second International Congress of Military Medicine and Pharmacy, Rome, May–June, 1923. By William Seaman Bainbridge, Commander, M.C., U.S.N.R.F., 148 p. (*The Military Surgeon, Washington, 1925.*)

The book is reprinted from several articles recently appearing in *The Military Surgeon.* The author had previously reported the proceedings of the First International Congress of Military Medicine and Pharmacy held in Brussels, 1921, in an article published in the United States Naval Medical Bulletin for 1922. These two Reports, as stated by Admiral Stitt in a paragraph of introduction to the present volume, form a valuable contribution to the medical records of the World War.

Among the subjects discussed by the delegates are the following: Medical organization of the army in war time; evacuation of the wounded, sick and gassed; organization of hospital services; selective distribution of casualties; the various phases of military hygiene; coöperation of civil and military authorities in the prevention of disease; military gymnastics; disinfection and disinfestation; trench foot; venereal disease; medical aspects of poison gas; abnormal individuals, degenerates and other mental defectives; psychoses and neuroses; graded fitness; tuberculosis as a war time problem; army laboratories; treatment of compound fractures and infected joints; gas gangrene; treatment of chest wounds; craniocerebral gunshot wounds; abdominal wounds; plastic surgery; and war pensions.

Although the discussion covers a wide range of subjects, one at once thinks of several notable omissions from the program, such as trench fever, war nephritis,

soldier's heart, surgical shock and primary suture.

However, as the author points out, the chief value of such a Congress is not in the range or thoroughness of the subjects discussed, but in the friendly intermingling of the delegates from the twenty-nine countries represented. Such sociable intercourse between the representatives of different nations is the surest means of international understanding, and the best safeguard against future wars.

A. R. K.

Fasting and Undernutrition, A Biological and Sociological Study of Inanition. By SERGIUS MORGULIS. XIII and 407 pp. $5.00. (*New York, E. P. Dutton and Co., 1923.*)

The widely spread famines and near-famines following in the immediate wake of the Great War forcibly brought the problems of starvation and undernutrition to the foreground in the scientific as well as the lay public interest. Professor Morgulis's book comes very opportunely as an excellent critical analysis and epitome of practically all of our more or less exact knowledge on this subject.

Under the main topic of physiological inanition the author presents a correlation of studies pertaining to hibernation, metamorphosis, and to inanition during the sexual activities of some animals, such as the salmon. Then follow very thorough discussions of the experimental forms—partial inanition, covering the subjects of oxygen starvation, water starvation, lack of minerals, proteins, fats, carbohydrates and vitamins in the diet; chronic inanition; and finally, of the relation of inanition to growth. Each of these topics is presented from the standpoint of chemical, physiological and morphological changes observed in the organism.

The voluminous literature is covered with a truly striking thoroughness, and the well arranged bibliography contains over 1200 references. The manner of presentation is simple and clear, and the author is to be commended on the excellence of his style, both of which qualities are not too often encountered in the scientific treatises of our day. The typography and general appearance of the book leave little room for criticism.

W. A. P.

The Medical Aspects of Chemical Warfare. By EDWARD B. VEDDER, Lt. Col., M.C., U.S.A. 327 pp. $6.50. (*The Williams and Wilkins Co., Baltimore, 1925.*)

This book is a masterful presentation of the various medical and semi-medical aspects of gas warfare. Its value is greatly enhanced by profuse illustrations. The subject matter includes the physics and chemistry of war gases; meteorology; the toxicology and pathology of the various war gases; their clinical aspects, including treatment; and the ultimate results of gas poisoning. Other problems such as individual protection, collective protection, and the field organization of the Medical Department for the treatment and transportation of gassed patients are likewise dealt with. There is also a chapter on the chlorine treatment of certain respiratory diseases. Lieutenant-Commander D. C. Walton, M.C., U.S.N., has contributed a chapter on the "Naval Medical Aspects of Chemical Warfare."

The publication of this book has fulfilled a real need for the Medical Officer of the Army or Navy, and for the Chemical Warfare Service. Heretofore, in order to obtain anything like a comprehensive knowledge of the medical problems involved in chemical warfare, it was necessary to wade through a mass of publications and unpublished government reports (many of which are difficult to obtain) sufficient to discourage him at the start, and at best calculated to make his task a very arduous one.

Colonel Vedder has collected together all the salient facts in a book of convenient size. In addition to being comprehensive, it has the great added value of being written in a clear, forceful, graphic style that presents the subject matter in a most pleasing manner.

A. R. K.

The Action and Uses in Medicine of Digitalis and Its Allies. By ARTHUR R. CUSHNY. 303 pp., $6.00. (*Longmans, Green and Co., 1925.*)

Several excellent reviews of different

phases of the action of digitalis have appeared in the last few years but from these this book differs fundamentally. It is not a mere review but a masterly record of the experiments, experience and thought of a man who has been interested in the pharmacology of digitalis for some thirty years and who has been intimately associated, from the very start with the clinical advances in methods of heart study, the polygraph and the electrocardiograph, which have done so much to unravel the therapeutic action of digitalis. It is a delight to those especially interested in this subject to have the views of so eminent an authority as Professor Cushny collected in this manner together with such a comprehensive and well arranged bibliography. It will be found by the general reader to be most clearly and interestingly expressed, and fully illustrated, and should be of great value to the practitioner of medicine.

The book begins with a short description of the sources of members of the digitalis group, and a description of the active principles. An interesting discussion of the chemistry of these active principles follows, and of the relationship of their physical properties to cardiac action.

The frog's heart is dealt with in detail, beginning with a historical sketch of the work and a discussion of the action in relation to the nervous mechanism, rhythm and conductivity, changes in excitability, action in the development of spontaneous beats, contraction and relaxation, the work done, and finally, a comparison of the action of the frog's heart with that of the hearts of other cold-blooded animals.

The effect on the mammalian heart is next taken up in detail, and each step very clearly illustrated with curves obtained from actual experiments. The two phases of digitalis action are discussed; in the first the effect of the increased contractility and inhibition, and in the second the impairment of conduction and the development of spontaneous rhythm in different parts of the heart leading to ectopic beats, ectopic rhythms and fibrillation.

In the next chapter the effect of digitalis on the blood pressure is discussed; its action on the heart, cardiac output, vascular changes in general, and in the different vascular beds is dealt with. Following this, the local irritant, anaesthetic and emetic action are taken up, as well as its effect on the respiration, kidney, central nervous system and muscular tissue, and the susceptibility of different animals is discussed. The absorption, distribution in the body and cumulative effects are then dealt with, and the last two chapters devoted to a most interesting survey of the therapeutic uses of digitalis and the methods of administration. The book contains 279 pages with 77 illustrations and 559 references to the literature.

P. D. L.

Gynecology. By WILLIAM P. GRAVES. 936 pp. Third edition. $9.00. (*W. B. Saunders Co., 1923.*)

Dr. Graves has made his good book even better by this third revision. The reading matter is well arranged and amply illustrated. There is a complete index which gives the reader immediate reference to any subject he wishes to look up. It would be difficult to criticise this book adversely; and the medical student, the general practitioner and the trained gynecologist will find it of great value.

There are, however, a few pages which we should like Dr. Graves to examine before he completes his next revision, although possibly by that time we ourselves may have changed our own opinions. I refer particularly to some of his references to "chronic interstitial endometritis" and the illustration on page 255. It does not seem that he is quite as clear as he might be in distinguishing genuine endometritis from a process which is apparently not inflammatory and which we call "hyperplasia of the endometrium." This is a clear cut clinical and pathological entity and deserves mention.

It is always easier to criticise a book than to write one. In this case, the task of the critic is unusually difficult, for Dr. Graves' experience and scientific acquirements have made this almost a model textbook.

L. R. W.

Surgical Infections of the Female Urethra and Cervix. By FRANK KIDD and A.

MALCOLM SIMPSON. 191 pp. $2.50. (*Oxford University Press, 1924.*)

This book is based on the experience obtained by the authors in a large public female venereal clinic in London. As it is intended for beginners, it starts with fundamental principles and gradually leads the reader more deeply into the special features of the work.

In general, one can say that the experience of these men is well worth reading. It is easy to see, however, that their viewpoint is not so wide as that of the trained gynecologist; their previous experience had been limited entirely to the treatment of venereal diseases in the male. As a consequence, their conclusions concerning certain gynecological questions are somewhat immature and show a lack of familiarity with the points at issue. Certain statements in this book, therefore, should be accepted with caution.

On the whole, however, the book is practical, concise, and contains much valuable information.

L. R. W.

Des Kindes Ernährung, Ernährungsstörungen und Ernährungstherapie. PROF. AD. CZERNY u. PROF. A. KELLER. $5.40. (*Franz Deuticke, Leipzig u. Wien.*)

The second edition of the first volume of this well known book has recently appeared much to the satisfaction of all students of pediatrics. The first edition was exhausted more than fifteen years ago and copies have been extremely difficult to obtain. This was sufficient reason for a reprinting, but the great increase in the knowledge of the nutrition of children and the nutritional diseases has also rendered such a course necessary.

It is no over-statement to say that this book is one of the most important for pediatrists ever published. It contains, beside the views of the authors, an extraordinary amount of accurately quoted information critically reviewed from a reasonably impartial viewpoint. No book in any language could replace it. It has occupied and still holds a really unique position in the medical literature of infancy and childhood.

J. H.

The Nature of Disease. By J. E. R. McDONAGH, F. R. C. S. 327 pp. 63 shillings. (*William Heinemann, Ltd., 1924.*)

The title of this book is somewhat misleading. From the cover, one might be led to expect, perhaps, a general treatise on the pathological aspects of disease. Instead, one finds a discussion of a variety of questions that have been the subject of study by the author and his associates. These topics range from that of the life cycle of the Spirochaeta pallida to the coagulation of the blood, the chemistry of staining, and the mechanism of the action of poisons upon the body. In general, an attempt is made to bring together under the aegis of physical chemistry many of the phenomena of physiology, pathology, immunology, and chemotherapy. The reviewer is not in a position to criticise the physical chemistry of the author, but he is surprised to find that it is possible to write so exhaustively on physical-chemical reactions without the use of equations bearing the letter k. It might not be amiss to quote two sentences from the work. One finds such gems as this: "Many cases of sleeplessness are due to the protein particles being poor in electricity. Even the behavior of the individual is moulded by the electronic activity of the protein particles." Again: "The treatment of disease rests in choosing the right drug at the right time, the right time meaning a correct divination of the loss of electricity sustained by the protein particles."

Part of the book is taken up with a presentation anew of the author's work on the life cycle of the Spirochaeta pallida. While most workers with this organism would doubtless admit that there may very well be a phase in the development of this organism which is quite different from the form which is familiar to all, still, the evidence which McDonagh has brought forward in support of such a view is not convincing in its present form.

On the whole, the book is unique and extraordinary from many points of view. Just what purpose it serves is not clear. It has a wealth of expensive cuts.

A. M. C.

Handbook of Bacteriology. (For Students and Practitioners of Medicine.) By JOSEPH W. BIGGER, M.D. 413 pages. $5.00. (*Wm. Wood and Co., New York, 1925.*)

In the preface the author states that students frequently complain of the length of textbooks with the consequent difficulty of sorting the essential from the mass of material presented and that as a result of such a presentation, they frequently rely upon "cram" books. He has written what is ostensibly a textbook but which in reality is a "cram" book dignified by publisher's practice and good presentation by the author of the commonly accepted and dogmatic teachings of bacteriology. For the most part the controversial ideas are omitted or simply mentioned in passing.

The book divides itself into three parts, technique and apparatus, theory underlying the preceding, and determinative bacteriology. In the first portion the working tools and tricks of the trade are described. The complement fixation reaction and other experiments are described as to technique in much the way in which the student performs the test in the laboratory. In the second part, the theoretical portion of the subject is presented as a result of this division in a small amount of space. The third part is devoted to a brief description of the various species of bacteria, higher fungi and animal parasites which have a pathological significance. It is interesting to note that the names recommended by Bergey's Manual have been added to the more commonly used terms.

To those interested in the embalmed knowledge of bacteriology as, for example, beginning students rushing through a three months' course for which compactness is essential, to those interested in passing examinations and to those who wish to refresh a hazy memory with fundamental facts, the book is to be recommended. The author has apparently accomplished his stated purpose.

E. L. B.

The Physiology of Mind. By FRANCIS X. DERCUM. 2d edit., 287 pp. $3.50. (*W. B. Saunders Co., Philadelphia and London, 1925.*)

This little volume opens with an interesting summary of the more recent neurological biology of Parker and Herrick, but shortly departs to attempt to explain all of the field of psychology and psychiatry by the very speculative hypothesis of "neuronic retraction." The author is very dogmatic in the field of thalamic and striatal localization—a dogmatism which our present knowledge hardly justifies. The vicious attack upon Freud in the appendix is an unfortunate addition.

L. B. H.